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Application of Fourier transform-second-harmonic generation imaging to the rat cervix

T.Y. LAU¹, H. K. SANGHA¹, E. K. CHIEN², B. L. MCFARLIN³, A. J. WAGONER JOHNSON¹, and K. C. TOUSSAINT JR.^{1,*}

¹ Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

² Alpert Medical School of Brown University and Women and Infants Hospital of Rhode Island, Providence, Rhode Island, USA

³ Department of Women, Children and Family Health Science, University of Illinois at Chicago, Chicago, Illinois, USA

SUMMARY

We present the application of Fourier transform-second-harmonic generation (FT-SHG) imaging to evaluate the arrangement of collagen fibers in five non-pregnant rat cervixes. Tissue slices from the mid-cervix and near the external orifice of the cervix were analyzed in both two-dimensions (2D) and three-dimensions (3D). We validate that the cervical microstructure can be quantitatively assessed in three dimensions using FT-SHG imaging, and observe collagen fibers oriented both in and out-of-plane in the outermost and the innermost layers, which cannot be observed using 2D FT-SHG analysis alone. This approach has the potential to be a noninvasive method for measuring progressive changes in collagen organization during cervical remodeling in humans.

Keywords

Second-harmonic generation; Fourier analysis; rat cervix

INTRODUCTION

Fifteen million babies worldwide are born premature, or before 37 completed weeks of gestation, each year (March of Dimes *et al.*, 2012). In the United States, premature birth is the second leading cause of infant mortality and the leading cause of infant mortality for African Americans (March of Dimes, 2009). Preterm birth is also linked with debilitating morbidity and has long-term effects including cardiovascular disease, hypertension, diabetes, dyslipidemia, neurocognitive disorders, respiratory conditions and cancer (Goldenberg *et al.*, 2008; Spong, 2007). Significant improvements could be made in the incidence of in-hospital mortality (reduction from 8.1% to 0.4%), cerebral palsy (reduction from 6.3% to 0.7%) and respiratory distress syndrome (reduction from 44% from 3%) if birth could be delayed from 30 to 34 weeks of gestation (Marret *et al.*, 2007). While advances have been made in recent decades in caring for premature infants, there has been very little change in the incidence of preterm birth in the last 40 years (Martin *et al.*, 2009). Mainstream clinical interventions aimed at stopping the uterus from contracting during preterm labor are ineffective for reducing the incidence of preterm birth (McFarlin, 2005).

* Corresponding author: Dr. K. C. Toussaint Jr., Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA. Tel: 217-244-4088; ktoussai@illinois.edu.

To this end, an improved understanding of the properties of the cervix could facilitate the development of appropriate intervention strategies to mitigate this problem.

The cervix has dual functions during pregnancy. Early in pregnancy it is firm in order to maintain pregnancy. Ultimately, the cervix softens to allow for the fetus to pass through the birth canal (House & Socrate, 2006; Myers *et al.*, 2008; Timmons *et al.*, 2010). This dramatic change in stiffness owes to significant microstructural and compositional changes of the cervix, which occur during a *cervical remodeling* phase that begins several months prior to contractions of the uterus (House & Socrate, 2006; Timmons *et al.*, 2010). During this period, the cervix transforms from a dense fibrous tissue with an organized network of collagen fibers to a soft distensible tissue through a process of collagen disorganization and decreased collagen concentration (House *et al.*, 2009; Myers *et al.*, 2009; Read *et al.*, 2007). In order to observe changes in collagen morphology during cervical remodeling, imaging techniques, such as X-ray diffraction (Aspden, 1988), polarized light microscopy (Yu *et al.*, 1995), electron microscopy (Yu *et al.*, 1995), and second-harmonic generation (SHG) microscopy (Akins *et al.*, 2010; Myers *et al.*, 2008; Zhang *et al.*, 2012) were used in previous studies. However, X-ray diffraction and electron microscopy expose tissues to ionizing radiation, while the latter and polarized light microscopy often require exogenous staining for contrast, which may not preserve the original microstructure of the tissue. In addition, a methodology that quantifies the changes in the organization of collagen fibers during cervical remodeling has not been reported. We propose the potential application of Fourier transform-second-harmonic generation (FT-SHG) imaging, which uniquely combines SHG imaging with spatial Fourier analysis, to noninvasively and quantitatively detect microstructural changes during cervical remodeling in 3D with high specificity to collagen fibers.

SHG is a nonlinear optical process wherein two input photons of the same frequency are simultaneously annihilated in a non-centrosymmetric medium upon which a single photon with twice the frequency is emitted at the output. Type I collagen, the most abundant fibrillar collagen in the human body, can emit a relatively strong SHG signal when illuminated by a high intensity optical field. Therefore, in an imaging modality, high contrast images of type I collagen fibers can be obtained without the need for staining with exogenous dyes (Campagnola *et al.*, 2002; Rao *et al.*, 2009). In addition, the intrinsic confocality of SHG microscopy, a result of the nonlinear process, permits imaging of samples in three dimensions (Campagnola *et al.*, 2002; Lau *et al.*, 2012; Nishino *et al.*, 2009; Pena *et al.*, 2007). In recent years, there has been much effort in the development of quantitative SHG microscopy, such as FT-SHG, whereby the unique process of SHG enables quantitative information of a collagen-based, tissue under study to be extracted readily when combined with spatial harmonic analysis. Our previous studies have demonstrated the utility of this technique in quantifying collagen fiber organization through the use of such metrics as orientation isotropy and the spatial frequency based on both 2D and 3D SHG images (Ambekar *et al.*, 2012; Lau *et al.*, 2012). Such metrics have been shown to permit the assessment of differences in spatial arrangement of collagen fibers between healthy and injured tendon tissues (Sivaguru *et al.*, 2010). FT-SHG has also been applied to determine dissimilarities in collagen fiber organization in human breast tissues at different pathological stages (e.g., hyperplasia, dysplasia, and malignant) (Ambekar *et al.*, 2012).

In this paper, we apply FT-SHG to quantitatively evaluate the microstructure of the cervix. Section 2 describes our methodology, including sample preparation, image acquisition, and image analysis. In section 3, we discuss the application of FT-SHG to cervical tissue from non-pregnant rats. We conclude in section 4 with a summary and a brief discussion of the

significance of this methodology to detect microstructural changes during cervical remodeling.

METHODS

Sample preparation

Cervical tissue was harvested from five non-pregnant rats using a protocol approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. Harvested samples were embedded in OCT blocks and stored at -80°C until sectioning. 50- μm -thick tissue sections, perpendicular to the cervical canals, were obtained proximal to the external orifice of the cervix (external os) and near the mid-cervix (half-way between the internal and external cervical os), as shown in Figure 1, for each embedded sample using a Leica CM3050 cryostat (Leica Microsystems Inc., Buffalo Grove, IL, USA).

Image acquisition

Epi-detected SHG images of cervical sections were acquired using a Zeiss LSM 710 confocal microscope (Carl Zeiss Microscopy, Maple Grove, MN 55369, USA) coupled with a femtosecond light source (Newport Corporation, Irvine, CA, USA) centered at 780-nm wavelength. The incident beam was circularly polarized to facilitate emission of SHG signals from collagen fibers at all orientations. In order to minimize refractive index mismatch between the sample and the imaging medium, a 1.2 numerical-aperture (NA) water immersion objective lens (Carl Zeiss Microscopy, Maple Grove, MN, USA) was chosen for both sample illumination and signal collection. The combination of a short-pass 760-nm filter followed by a 390/20-nm band-pass filter (Newport Corporation, Irvine, CA, USA) was placed between the objective and a GaAsP-NDD detector (Carl Zeiss Microscopy, Maple Grove, MN, USA) to extract the backscattered SHG signal.

Lateral scanning step sizes ranging from 208 nm to 2.1 μm were adapted for imaging rat cervical tissues at different scales. For imaging whole transverse slices of rat cervix, SHG images were acquired by raster scanning the incident beam at a lateral step size of 2.1 μm using a galvoscaner. A lateral step size of 208 nm was used to obtain higher resolution images of the selected characteristic regions. 3D image stacks of the characteristic regions were achieved by sampling at a lateral step size of 208 nm and an axial step of 210 nm. Using similar step sizes in both lateral and axial directions can avoid interpolation between consecutive images as interpolation may introduce uncertainties. Among the five rat cervixes used in this study, three were used for 2D analysis and two were used for 3D analysis. A complete image of each transverse slice was taken for all rat cervixes.

Image analysis

To quantitatively assess the arrangement of collagen fibers in cervical tissue, both 2D and 3D FT-SHG imaging was carried out. FT-SHG is a well-established imaging method that has been applied to various biological structures, including cornea (Ambekar & Toussaint, 2011), tendon (Sivaguru *et al.*, 2010), and bone (Ambekar, *et al.*, 2012), and a detailed description of the technique can be found elsewhere (Lau *et al.*, 2012; Rao *et al.*, 2009). Briefly, Fourier transform (FT) analysis was performed on a gridded (2D) SHG image as well as (3D) image stacks; each region bounded by the grid was examined individually. The grid size was carefully chosen such that each bounded region was small enough to describe the local orientation of collagen fibers while avoiding common errors due to pixelation. Each region that displayed a dominant orientation in the frequency spectrum was labeled as anisotropic and assigned a preferred orientation, whereas regions that lacked prominent orientations were labeled as isotropic. In addition, regions with low or negligible SHG

intensity did not provide representative information of orientation isotropy; those regions were consequently labeled as dark regions.

For quantitative assessment, a circular histogram summarizing preferred orientations of anisotropic regions and a histogram of labeled regions were generated for each analyzed image. The respective circular variance of each region was calculated as well in order to provide quantitative measures of the orientation isotropy of the circular histogram. The circular variance, a dimensionless measurement of the spread of orientation (Fisher, 1993; Jammalamadaka & Sengupta, 2001), has a value between 0 and 1; the smaller the circular variance, the tighter the distribution of preferred orientation.

RESULTS AND DISCUSSION

2D FT-SHG

Figure 2(a) is a representative image of a tissue slice obtained from the mid-cervix. The FT analysis shows circumferentially aligned collagen fibers that are approximately symmetric about both the sagittal and coronal planes. The loop containing the aligned fibers has its center at the intersection of these planes. The analysis also shows that areas adjacent to the highly aligned fibers and between the cervical canals are predominantly isotropic [Fig. 2(b)]. From the same sample, a tissue slice obtained from near the external os of the cervix displays similar arrangement of collagen fibers [Figs. 2(c) and 2(d)]. Likewise, tissue slices from four additional rat cervix samples are found to be similar in their spatial arrangement of collagen fibers at both longitudinal locations. This initial evaluation of the rat cervix indicates symmetries and consistency in fiber organization on the transverse plane and at two different depths in the organ, respectively. Therefore, it provides a basis for us to select characteristic regions for a more detailed evaluation of collagen fiber organization.

Given the differences in collagen fiber organization in the region between the cervical canals and in the regions containing the circumferentially oriented fibers, three characteristic regions in both the mid-cervix tissue slice and external os slice were identified for imaging and analysis at a lateral step size of 208 nm (number of animals, $n = 3$) [Fig. 3]. The circular histograms of preferred orientation of anisotropic regions are shown alongside their corresponding SHG images in Fig. 3, and the quantity circular variance is a measure of the spread in the orientation. In addition, the histogram of labeled regions provides information about the contribution of each labeled region in each SHG image. In brief, dark regions represent areas with negligible amount of SHG signal, and isotropic regions and anisotropic regions indicate areas where fibers are oriented randomly, or along a pronounced preferred orientation, respectively. Averaging over the six tissue slices analyzed, the circular variances of the region between the cervical canals, and the two regions showing predominately circumferentially-oriented fibers near the coronal plane and the sagittal plane are 0.187, 0.132, and 0.0898, respectively. In the same order, the ratio of anisotropic regions to isotropic regions are 1.40 (standard deviation = 1.27), 1.87 (standard deviation = 0.614), and 1.61 (standard deviation = 0.297).

The region between the cervical canals, proximal to the intersection of the sagittal and coronal planes (region 1), has a higher circular variance relative to the regions containing the circumferentially oriented fibers. The higher circular variance indicates that the organization of collagen fibers in this region is more isotropic, i.e., the collagen fibers are less aligned, compared with the regions containing the circumferentially oriented fibers. In fact, the SHG image of the central region displays an interwoven network of collagen fibers in many different orientations [Fig. 2(a)]. Consistently, the FT-SHG analysis shows that isotropic regions and anisotropic regions are randomly distributed across the central region [Fig. 3(a)]. For the regions containing the circumferentially oriented fibers, results of the FT-SHG

analysis highlight the group of circumferentially oriented fibers mentioned in the initial evaluation [Figs. 3(b) and 3(c)]. For both images, their circular histograms of orientation of anisotropic regions display relatively low circular variances, which indicate that collagen fibers along the circumferential loop are highly aligned. Similar to Figure 2(a), areas in the immediate surroundings of the highly aligned fibers are relatively isotropic for both regions. By observation, the regions that are highly anisotropic follow a circumferential loop around the cylindrical cervix. Therefore, regions 2 and 3 are approximately equivalent to each other.

Such interesting fiber arrangement divides the rat cervix into three distinct sections: a section with highly aligned collagen fibers sandwiched between two isotropic sections [Fig. 4]. In addition, the isotropic sections also points to a potential 3D fibrillar structure present in the rat cervix. Our previous report on 3D FT-SHG analysis indicated that the regions labeled as isotropic by the 2D FT analysis may potentially contain out-of-plane fibers (Lau *et al.*, 2012). Out-of-plane or oblique, aligned fibers appear as clusters of bright spots due to the fact that each 2D image can only capture a small portion of their lengths. In comparison, the 3D FT-SHG analysis avoids such ambiguity by taking into account multiple images throughout the depth of the tissue slices. Therefore, the aforementioned 3D analysis may provide a more comprehensive evaluation of the rat cervix.

3D FT-SHG

Figure 5 displays results of the 2D and 3D FT-SHG analysis of a tissue slice obtained from the mid-cervix. The 2D analysis of the SHG image of the whole tissue slice shows the same pattern of collagen fiber organization described previously [Fig. 5(b)]. Subsequently, the analysis focuses on analyzing the characteristic regions from a 3D perspective. Despite the high orientation isotropy observed from the 2D image of the region between the canals, there is a significant number of collagen fibers oriented obliquely to the image plane according to the 3D rendered image of the central region as well as the 3D FT-SHG analysis [Figs. 5(c) and (d)]. For the region outside of the cervical canals, labeled as region 2 in Fig. 5(a), a substantial number of out-of-plane fibers are present at locations where the 2D analysis identified as isotropic [Figs. 5(e) and (f)]. Similar arrangement of collagen fibers is also found in another sample of rat cervix. Therefore, the 3D analysis provides us with not only similar information about the preferred orientation of in-plane fibers as the 2D analysis, but is more revealing with respect to the fiber orientation in the predominately isotropic areas labeled by the 2D analysis. As shown in Fig. 6, for some of those isotropic regions, there exist out-of-plane fibers as indicated by the 3D analysis.

The 3D analysis is in agreement with previous models of the rat cervix. It has been formerly established that rat cervix consists of three concentric layers around each cervical canal: a layer of circumferentially aligned fibers, parallel to the transverse plane, sandwiched between two layers composed of predominately longitudinal fibers (Yu *et al.*, 1995). This aforementioned structure gives the rat cervix the shape and required mechanical properties to fulfill its role as part of the reproductive system. The human cervix is also arranged similarly except that there is only one cervical canal (Myers *et al.*, 2009). Importantly, the shared structural characteristics between rat and human tissue signify potential application of our technique to assess human cervical tissue.

CONCLUSION

SHG imaging allows us to obtain high contrast images of collagen fibers in the rat cervix tissue without the need for exogenous staining. Using FT analysis, we are able to evaluate SHG images of rat cervixes from both 2D and 3D perspectives. The FT-SHG analysis of the rat cervix shows that the collagen fiber organization varies at different locations on the transverse plane, but remains relatively constant along the longitudinal direction. According

to the 3D FT analysis, there is a pronounced loop of circumferentially oriented fibers in the rat cervix, but collagen fibers in the immediate surroundings of this area and in the region between the cervical canals are predominantly aligned out-of-plane. The observed structure of the rat cervix is similar to the human cervix model proposed by previous studies. Based on the similarity between human and rat cervixes 3D FT-SHG has potential to become a noninvasive approach for detecting early cervical tissue microstructural changes associated with cervical remodeling in human pregnancy for predicting preterm birth.

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REFERENCES

- Akins ML, Luby-Phelps K, Mahendroo M. Second harmonic generation imaging as a potential tool for staging pregnancy and predicting preterm birth. *J. Biomed. Opt.* 2010; 15:26020.
- Ambekar R, Chittenden M, Jasiuk I, Toussaint KC Jr. Quantitative second-harmonic generation microscopy for imaging porcine cortical bone: Comparison to SEM and its potential to investigate age-related changes. *Bone*. 2012; 50:643–650. [PubMed: 22155019]
- Ambekar R, Lau T-Y, Walsh M, Bhargava R, Toussaint KC. Quantifying collagen structure in breast biopsies using second-harmonic generation imaging. *Biomed. Opt. Express*. 2012; 3:2021–2035. [PubMed: 23024898]
- Ambekar, R.; Toussaint, KC. *Frontiers in Optics*, p. FTuQ2. Optical Society of America; 2011. Investigation of Collagen Fiber Organization in Cornea and Sclera using Quantitative SHG Microscopy..
- Aspden RM. Collagen Organisation in the Cervix and its Relation to Mechanical Function. *Coll. Relat. Res.* 1988; 8:103–112. [PubMed: 3378391]
- Campagnola PJ, Millard AC, Terasaki M, Hoppe PE, Malone CJ, Mohler WA. Three-Dimensional High-Resolution Second-Harmonic Generation Imaging of Endogenous Structural Proteins in Biological Tissues. *Biophys. J.* 2002; 82:493–508. [PubMed: 11751336]
- Fisher, NI. *Statistical analysis of circular data*. 1st ed.. Cambridge University Press; Cambridge: 1993.
- Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *The Lancet*. 2008; 371:75–84.
- House M, Socrate S. The cervix as a biomechanical structure. *Ultrasound Obstet. Gynecol.* 2006; 28:745–749. [PubMed: 17063451]
- House, Michael; Kaplan, DL.; Socrate, Simona. Relationships Between Mechanical Properties and Extracellular Matrix Constituents of the Cervical Stroma During Pregnancy. *Semin. Perinatol.* 2009; 33:300–307. [PubMed: 19796726]
- Lau TY, Ambekar R, Toussaint KC. Quantification of collagen fiber organization using three-dimensional Fourier transform-second-harmonic generation imaging. *Opt. Express*. 2012; 20:21821–21832. [PubMed: 23037302]
- March of Dimes. *Data Book for Policy Makers: Maternal, Infant, and Child Health in the United States*. Atlanta, GA: 2009.
- March of Dimes, PMNCH, Save the Children & World Health Organization. *Born Too Soon: The Global Action Report on Preterm Birth*. Geneva: 2012.
- Marret S, Ancel P, Marpeau L, Marchand L, Pierrat V, Larroque B, Foix-L'Hélias, Thiriez G, Fresson J, Alberge C, Rose J, Matis J, Breart G, Kaminski M. Neonatal and 5-Year Outcomes After Birth at 30–34 Weeks of Gestation. *Obstet. Gynecol.* 2007; 110:1172. [PubMed: 17978139]
- Martin JA, Hamilton B, Venture S, Menacker F, Park M, Sutton P. *Births: final data for 2001*. National Statistics Report. 2009; 51:1–104.

- McFarlin, B. PhD Thesis. University of Illinois at Chicago; Chicago: 2005. Quantitative Ultrasound Assessment of the Rat Cervix..
- Myers KM, Paskaleva AP, House M, Socrate S. Mechanical and biochemical properties of human cervical tissue. *Acta Biomater.* 2008; 4:104–116. [PubMed: 17904431]
- Myers K, Socrate Simona, Tzeranis D, House Michael. Changes in the biochemical constituents and morphologic appearance of the human cervical stroma during pregnancy. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2009; 144(Supplement 1):S82–S89. [PubMed: 19303693]
- Nishino Y, Takahashi Y, Imamoto N, Ishikawa T, Maeshima K. Three- Dimensional Visualization of a Human Chromosome Using Coherent X-Ray Diffraction. *Phys. Rev. Lett.* 2009; 102:18101.
- Pena A-M, Fabre A, Débarre D, Marchal-Somme J, Crestani B, Martin J-L, Beaurepaire E, Schanne-Klein M-C. Three-dimensional investigation and scoring of extracellular matrix remodeling during lung fibrosis using multiphoton microscopy. *Microsc. Res. Tech.* 2007; 70:162–170. [PubMed: 17177275]
- Rao RA, Mehta MR, Toussaint KC. Fourier transform-second-harmonic generation imaging of biological tissues. *Opt. Express.* 2009; 17:14534–14542. [PubMed: 19687932]
- Read CP, Word RA, Ruscheinsky MA, Timmons BC, Mahendroo MS. Cervical remodeling during pregnancy and parturition: molecular characterization of the softening phase in mice. *Reproduction.* 2007; 134:327–340. [PubMed: 17660242]
- S. Rao Jammalamadaka Ambar Sengupta. *Topics in Circular Statistics.* World Scientific; Singapore: 2001.
- Sivaguru M, Durgam S, Ambekar R, Luedtke D, Fried G, Stewart A, Toussaint KC. Quantitative analysis of collagen fiber organization in injured tendons using Fourier transform-second harmonic generation imaging. *Opt. Express.* 2010; 18:24983–24993. [PubMed: 21164843]
- Spong CY. Prediction and Prevention of Recurrent Spontaneous Preterm Birth. *Obstet. Gynecol.* 2007:110.
- Timmons B, Akins M, Mahendroo M. Cervical remodeling during pregnancy and parturition. *Trends Endocrinol. Metab.* 2010; 21:353–361. [PubMed: 20172738]
- Yu SY, Tozzi CA, Babiarez J, Leppert PC. Collagen Changes in Rat Cervix in Pregnancy—Polarized Light Microscopic and Electron Microscopic Studies. *Proc. Soc. Exp. Biol. Med.* 1995; 209:360–368. [PubMed: 7638243]
- Zhang Y, Akins ML, Murari K, Xi J, Li M-J, Luby-Phelps K, Mahendroo M, Li X. A compact fiber-optic SHG scanning endomicroscope and its application to visualize cervical remodeling during pregnancy. *Proc. Natl. Acad. Sci.* 2012; 109:12878–83. [PubMed: 22826263]

Fig. 1. Schematic of the rat cervix. Transverse slices (perpendicular to the canals) were taken from the near the mid-cervix (half-way between the internal and external cervical os) and the external orifice of the cervix (external os). The rat cervix has two cervical canals.

Fig. 2.

(a) SHG image of a tissue slice obtained from the mid-cervix. Red boxes indicate the selected characteristic regions. (b) Results of FT-SHG analysis of (a). (c),(d) The SHG image and results of FT-SHG analysis of the tissue slice from near the external os of the same cervix sample. The scale is the same for (a-d) and indicated in (a).

Fig. 3.

Results of FT-SHG analysis of the characteristic regions indicated on Fig. 2(a). (a) The SHG image of the region between the cervical canals, proximal to the intersection of the sagittal and coronal planes, is overlaid with colored blocks indicating spatial locations of isotropic and dark regions, as well as arrows representing preferred orientations of anisotropic regions. A circular histogram summarizing the distribution of preferred orientation of the anisotropic regions and a histogram of labeled regions are generated for quantitative evaluation. The same analysis is shown for region 2 and region 3 labeled on Fig. 2(a), in (b) and (c), respectively. The coronal plane and the sagittal plane correspond to 0° and 90° on the circular histograms. Scale bar = $100\ \mu\text{m}$.

Fig. 4.

A representative illustration of the three sections, each with distinct fiber organization, for the region outside of the cervical canals (indicated as region 2 on Fig. 2(a)). The section sandwiched between two isotropic sections contains highly organized collagen fibers, which are part of the loop of aligned fibers mentioned in the initial evaluation. The isotropic sections are composed of a mix of randomly distributed isotropic and anisotropic regions.

Fig. 5.

Results of FT-SHG analysis of a tissue slice from the mid-cervix. Both (a) the SHG image and (b) the 2D FT analysis show a group of highly aligned circumferential fibers. In addition, the 2D FT analysis again highlights the predominantly isotropic region between the cervical canals. For this region between the cervical canals, (c) the 3D rendered model, and (d) the 3D FT analysis prove the existence of a 3D fibrillar network in this region. Similarly, out-of-plane fibers are present in the region outside of the cervical canals according to (e) the 3D rendered model of this region, and (f) the 3D FT analysis. Note that the regions in (c) and (e) measure $1062.5\ \mu\text{m} \times 212.5\ \mu\text{m} \times 23.5\ \mu\text{m}$. The blue and red arrows indicate in-plane and out-of-plane orientations, respectively. The scale is the same for (a) and (b) and indicated in (a).

Fig. 6.

A comparison between (a) 3D FT-SHG and (b) 2D FT-SHG on a region outside of the cervical canals. White lines and red lines in (a) represents, respectively, in-plane and out-of-plane preferred orientations calculated using 3D FT-SHG. Most of the out-of-plane fibers are located at the isotropic sections previously identified using the 2D analysis. The scale is the same for (a) and (b) and indicated in (a).