Optical diagnosis of dermatofibrosarcoma protuberans differentiated from dermatofibroma using non-linear optical microscopy

Wei Tian[1], Xiaoqin Zhu[2], Xiaomin Huang[2], Ying Huang[2], Yamin Lin[2], Yinghong Yang[3], Jianxin Chen[2], Lihang Lin[2]

[1]Department of Dermatology, Affiliated Union Hospital, Fujian Medical University, Fuzhou, Fujian 350001, China;
[2]Key Laboratory of OptoElectronic Science and Technology for Medicine of Ministry of Education, Fujian Provincial Key Laboratory of Photonics Technology, Fujian Normal University, Fuzhou, Fujian 350007, China;
[3]Department of Pathology, Affiliated Union Hospital, Fujian Medical University, Fuzhou, Fujian 350001, China.

To the Editor: Dermatofibrosarcoma protuberans (DFSP) is a locally aggressive mesenchymal neoplasm of the skin that can be misdiagnosed as dermatofibroma (DF) because of similar histological characteristics.[1-3] In this study, non-linear optical (NLO) microscopy was used for imaging unstained histological sections to make a differential diagnosis between DFSP and DF. This study aimed to demonstrate that label-free NLO imaging provides significant advantages over standard hematoxylin-eosin (H&E) staining and allows pathologists to increase the diagnostic accuracy by using a quantifiable methodology.

The study was approved by the Ethics Committee of the Affiliated Union Hospital of Fujian Medical University (No. 2021KY023). Dermatofibrosarcoma protuberans and DF tissue samples were obtained from the Affiliated Union Hospital of Fujian Medical University. Patients signed a waiver of informed consent for the use of archival tissue specimens. The NLO imaging system used in this study contained a high-throughput scanning inverted axiovert 200 microscope (LSM 510 META; Zeiss, Jena, Thuringia, Germany) and a mode-locked femtosecond Ti: sapphire laser (110 fs, 76 MHz), operating at 810 nm (Mira 900-F; Coherent, Santa Clara, CA, USA).

As observed from the large-area NLO image of normal skin, the microstructure and the distribution of collagen or elastic fibers vary in the superficial (region of interest, ROI 1), middle (ROI 2), and deep dermis (ROI 3) [Figure 1]. Collagen appears thin in the superficial dermis and becomes increasingly coarser in the deeper dermis. In contrast to normal skin, NLO images of DF revealed a hyperplastic epidermis with acanthosis, as well as widespread hyperpigmentation of the basal layer. DF often penetrates only ROIs 1 and 2. The collagen and the elastic fibers in ROI 3 are similar to those in normal skin. Elastic fibers are assembled in ROI 1, and few elastic fibers are observed in ROI 2. The structure of the collagen fibers is slightly different from those in normal skin. DF shows a smooth and clear margin that bulges into subcutaneous tissue. DF is characterized by poorly demarcated, dermal spindle proliferation, which is composed of a mixture of collagenous stroma, histiocytes, multinucleate giant cells, and blood vessels in varying proportions. All lesions displayed hyperplasia of the overlying epidermis and thickened collagen bundles in the dermis.

Dermatofibrosarcoma protuberans differed from DFs in both epidermal and dermal microstructures. The dermal location of DFSP lies within a narrow, tumor-free zone beneath the atrophic epidermis. The ill-defined cellular tumor is present within the deeper dermis, and there are uniform spindle cells that form storiform patterns. Moreover, DFSP infiltrates into the subcutaneous tissue with a typical honeycomb pattern, in which the neoplastic and the spindle-shaped cells extend between fat cells, and the spindle cell layers are oriented parallel to the skin surface.[4]

To quantitatively compare alterations in the distribution of cells and matrix, the spectra of the NLO signals from ROIs 1, 2, and 3 in the three samples were measured and normalized [Supplementary Figure 1, http://links.lww.com/CM9/A582]. Each spectrum showed a peak at 405 nm and a broad spectral feature characteristic of two-photon excited fluorescence (TPEF). The sharp peak at 405 nm represented the second-harmonic generation (SHG) signal emitted from polarizable collagen. The collagen peak demonstrated a quadratic dependence on the incident laser intensity and shifted with changes in laser frequency to remain at exactly half the excitation wavelength. Elastin

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Wei Tian and Xiaoqin Zhu contributed to the work equally.

Correspondence to: Dr. Lihang Lin, Department of Dermatology, Affiliated Union Hospital, Fujian Medical University, Fuzhou, Fujian 350001, China
E-Mail: 460879404@qq.com

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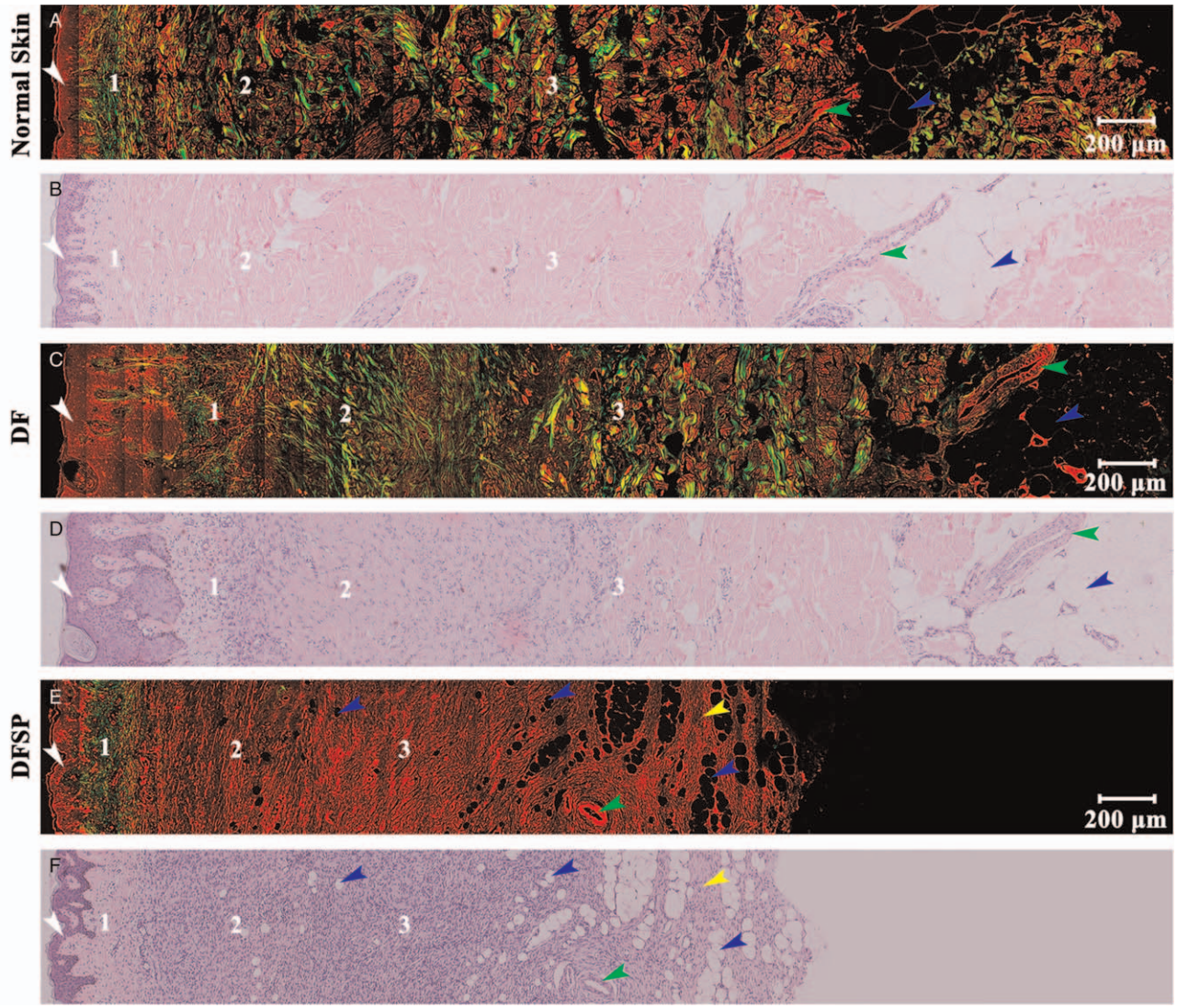

Figure 1: Representative large-area NLO images of normal skin (A), DF (C), and DFSP (E) revealing the tissue architectural changes from epidermis, dermis, to subcutaneous fat. The H&E images (B, D, F) correspond to the NLO images (A, C, E), respectively. White arrowheads: epidermis; green arrowheads: blood vessels; blue arrowheads: adipose cells; yellow arrowheads: spindle cell layers. ROIs 1, 2, and 3 are part of the superficial, middle, and deep dermis, respectively. DF: Dermatofibroma; DFSP: Dermatofibrosarcoma protuberans; NLO: Non-linear optical; ROI: Region of interest. Bar = 200 μm.

fluorescence was centered at approximately 500 nm, and intracellular fluorescence signals originating from nicotinamide dinucleotide (NADH) and flavin adenine dinucleotide (FAD) were centered at approximately 475 mm and 535 nm, respectively. Derived from the normalized spectra of the three tissues, the collagen-SHG signal intensity appears larger than the TPEF signals from other components in the dermis of normal skin. In the ROI 1 signal of the DF sample, TPEF signals are stronger mainly because of the concentration of elastic fibers.

In DFSP, TPEF signals from ROI 1 also seem to be stronger than those in normal skin. Most importantly, SHG signals from ROIs 1 and 2 are much weaker than those in DF or normal skin, owing to the loss of polarizable collagen bundles in the dermis located with the DFSP. This unique optical characteristic of DFSP is crucial for differentiating DFSP from DF. To quantitatively compare the intensities of SHG and TPEF signals, the SHG-to-TPEF index (STI) was evaluated by computing the ratio of (SHG – TPEF): (SHG + TPEF), based on the NLO spectra of normal skin, DF, and DFSP [Supplementary Table 1, http://links.lww.com/CM9/A582]. Except for ROIs 2 and 3 in DFSP, all other STIs were positive, which means that the SHG signal intensity is larger than the TPEF signal intensity. For DFSP tissues, the STI was -0.44 ± 0.21 and -0.42 ± 0.22 in ROIs 2 and 3, respectively, indicating that the SHG signal intensity was smaller than the TPEF signal intensity. It was shown that the loss of polarizable collagen bundles in the dermis of DFSP tissues, as well as established an effective method for identifying and locating the lesion of DFSP.

Magnified SHG/TPEF images from ROIs 1, 2, and 3 of the three tissue types need to be further examined for signals emitting from collagen, elastin, or other cellular structures. Cellular structures mainly included non-polarizable collagen fibers and cytoplasm and emitted much weaker TPEF signals compared with the elastic fibers. Based on these

differences, images were processed and segmented with the threshold value method to separate elastic fibers from other components as shown in Supplementary Figure 2, http://links.lww.com/CM9/A582, allowing clearer and easier observation and quantification of the elastic fibers. The images illustrate various features of collagen and elastic fibers in DF and DFSP that differ from those in normal skin. In normal skin, SHG images showed that collagen bundles are coarser from the superficial dermis throughout the deep dermis.

Similar to normal skin, DF is composed of thick, polarizable collagen bundles that emit strong SHG signals. However, collagen bundles in the superficial and middle dermis seem to be disrupted given the surrounding lesional cells, and the elastic fibers gather in the superficial dermis but are rarely found in the middle dermis. In DFSP tissues, both SHG signals from collagen bundles and TPEF signals from elastic fibers are observed in the Grenz zone. However, SHG signals were absent in the deeper dermis throughout the invaded subcutaneous tissue. Collagen bundles in DFSP lesions are non-polarizable and cannot produce SHG signals. Moreover, the collagen in the superficial dermis is thin and fragmented; the fragmented elastic fibers are found in the superficial dermis, and a large number of elastic fibers are observed in the middle dermis. In the middle dermis, coarser elastic fibers form a distinguishable network.

As mentioned above, DF and DFSP are composed of collagen and elastic fibers in varying proportions. To quantify the amount of matrix in different regions, the density of collagen and elastic fibers in normal skin, DF, and DFSP was measured [Supplementary Figure 3, http://links.lww.com/CM9/A582]. The density of collagen and elastic fibers was calculated by dividing the pixel number of collagen SHG signals or elastin TPEF signals by the total pixel number of the involved region. It was seen that the collagen densities of the superficial or ROIs 1 and 2 in normal skin were much higher than that in DF ($42.6 \pm 6.7\%$ *vs.* $14.1 \pm 5.8\%$ in ROI 1, $P < 0.001$; $46.5 \pm 5.2\%$ *vs.* $23.2 \pm 3.3\%$ in ROI 2, $P < 0.001$). The collagen density of ROI 3 was similar in normal skin and DF ($37.8 \pm 7.2\%$ *vs.* $37.6 \pm 4.6\%$, $P = 0.962$). These data confirm previous descriptions that DF often involves the papillary and reticular dermis. Furthermore, the collagen density of the superficial dermis in DFSP ($21.2 \pm 8.1\%$) is lower than in normal skin but somewhat higher than in DF, while the collagen density of the middle and deep dermis in DFSP was the lowest, at nearly 0 ($2.7 \pm 2.1\%$ and $0.4 \pm 0.2\%$) owing to the non-SHG signals of non-polarizable collagen.

Derived from Figure 1B, the density of elastic fibers in normal skin is similar across the layers ($3.3 \pm 0.7\%$, $4.3 \pm 2.8\%$, and $2.4 \pm 1.1\%$ in ROIs 1, 2, and 3, respectively). However, the elastin content varied among the layers in DF (ROIs 1, 2, and 3: $10.3 \pm 4.6\%$, $0.2 \pm 0.2\%$, and $2.6 \pm 1.4\%$, respectively) as well as in DFSP (ROIs 1, 2, and 3: $15.0 \pm 3.0\%$, $13.6 \pm 4.8\%$, and $0.5 \pm 0.3\%$, respectively). The density of elastic fibers in the superficial dermis in DF was significantly larger than in normal skin ($10.3 \pm 4.6\%$ *vs.* $3.3 \pm 0.7\%$, $P < 0.026$). The density of elastic fibers of the superficial dermis in DFSP was also significantly greater than that of normal skin ($15.0 \pm 3.0\%$ *vs.* $3.3 \pm 0.7\%$, $P < 0.001$). These observations are likely because of the assembled elastic fibers in the superficial dermis of DF and DFSP.

To conclude, we strongly believe that NLO microscopy has a promising future in distinguishing DFSP from DF tissues in a manner resembling traditional histological analysis, and could be used to conduct a retrospective study of DFSP in a large-scale study sample.

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Conflicts of interest

None.

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