Three-dimensional Skin Imaging Using the Combination of Reflected Confocal and Multiphoton Microscopy

Ming-Gu Lin[1], Wei-Liang Chen[1], Wen Lo[1], Hsin-Yuan Tan[2], Tsung-Hua Tsai[3], Shiou-Hwa Jee[4], Sung-Jan Lin[4#], Chen-Yuan Dong[1*]

[1]*Department of Physics, National Taiwan University, Taipei, Taiwan*
[2]*Department of Ophthalmology, Chang Gung Memorial Hospital, Linko, Taiwan*
[3]*Department of Dermatology, Far Eastern Memorial Hospital, Taipei, Taiwan*
[4]*Department of Dermatology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan*

ABSTRACT

Reflected confocal microscopy has been widely used in clinical application in dermatology. In recent years, multiphoton microscopy has also emerged as an important minimally invasive bioimaging technique for the skin. In this study, we combine reflected confocal microscopy and multiphoton microscopy for skin imaging. In the epidermis, reflected confocal signals are expected to help in delineating cell borders while multiphoton signals provide cytoplasmic morphologies. In the dermis, second harmonic generation signals provide the morphology of collagen fibers. When three-dimensional images are projected, the detailed distribution of cellular component and extracellular matrix in skin can be obtained. Properly developed, this technique is of great potential for in vivo clinical application.

Keywords: reflected confocal, multiphoton, microscopy, skin

Address correspondence to: #S. J. Lin at sjlin@ha.mc.ntu.edu.tw and *C. Y. Dong at cydong@phys.ntu.edu.tw

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1. Introduction

Reflected confocal laser scanning microscopy has been widely used in clinical diagnosis and biomedical researches recently.[1-4] This technique relies on signals that occur at interface of changing refractive indices for imaging. By adjusting the pinhole, the signals generated at the selected focal plane can be collected and detected. The advantages of reflected confocal laser scanning microscopy are the negligible photodamage and the optical sectioning ability. In recent years, another minimally invasive technique is multiphoton microscopy that has also been demonstrated to be a powerful modality for the biomedical research.[5-8] It is advantageous in that the point-like excitation volume yields high contrast images, the minimally excitation volume can reduce the overall photodamage, and the reduced scattering of near-infrared excitation photons allow deeper specimen imaging depth to be achieved.[9-12] In addition to multiphoton excitation, the nonlinear optical process of second harmonic generation (SHG) has also been offered to analyze structures with non-centrosymmetry. Several biological structures have been proven to be effective in producing the SHG signals, such as collagen, muscle fibers, and microtubules.[13,14] In this study, we attempt to image the three-dimensional structures of skin by using the combination of reflected confocal and multiphoton microscopy. The cellular components of skin can be more clearly delineated by the complementary information from autofluorescence (AF) and reflected confocal (RC) signals. We found that reflected confocal signals are limited in its ability to the dermal structures. However, this limitation can be compensated by the multiphoton images. The collagen of dermis can be identified by SHG signals. Our work demonstrates that the combination of the two imaging techniques can visualize the three-dimensional skin architecture in detail from the epidermis to the dermis.

2. Materials and method

Specimen preparation

A fresh skin specimen was used in this study. The experiment was performed within 24 hours after the skin was obtained from a cosmetic surgery. In our preliminary study, the thick stratum corneum in the specimen limited the imaging depth. In this study, tape stripping by adhesive tapes was performed prior to the imaging process. The area of skin specimen imaged was approximately 3 by 3 mm.

Reflected confocal and multiphoton microscopy imaging

The imaging system we used in this study is based on a commercial laser scanning microscope (Zeiss LSM 510 Meta). The instrumental setup is shown in Fig. 1. The reflected confocal and multiphoton imaging of the skin was performed by using the near-infrared excitation light from a titanium-sapphire laser pumped by a diode-pumped, solid state laser system. The multiphoton excitation wavelength was 780 nm and multiphoton autofluorescence and SHG signals from the skin specimen are acquired simultaneously. The reflected confocal and multiphoton images were separately acquired by multi-pass scanning.

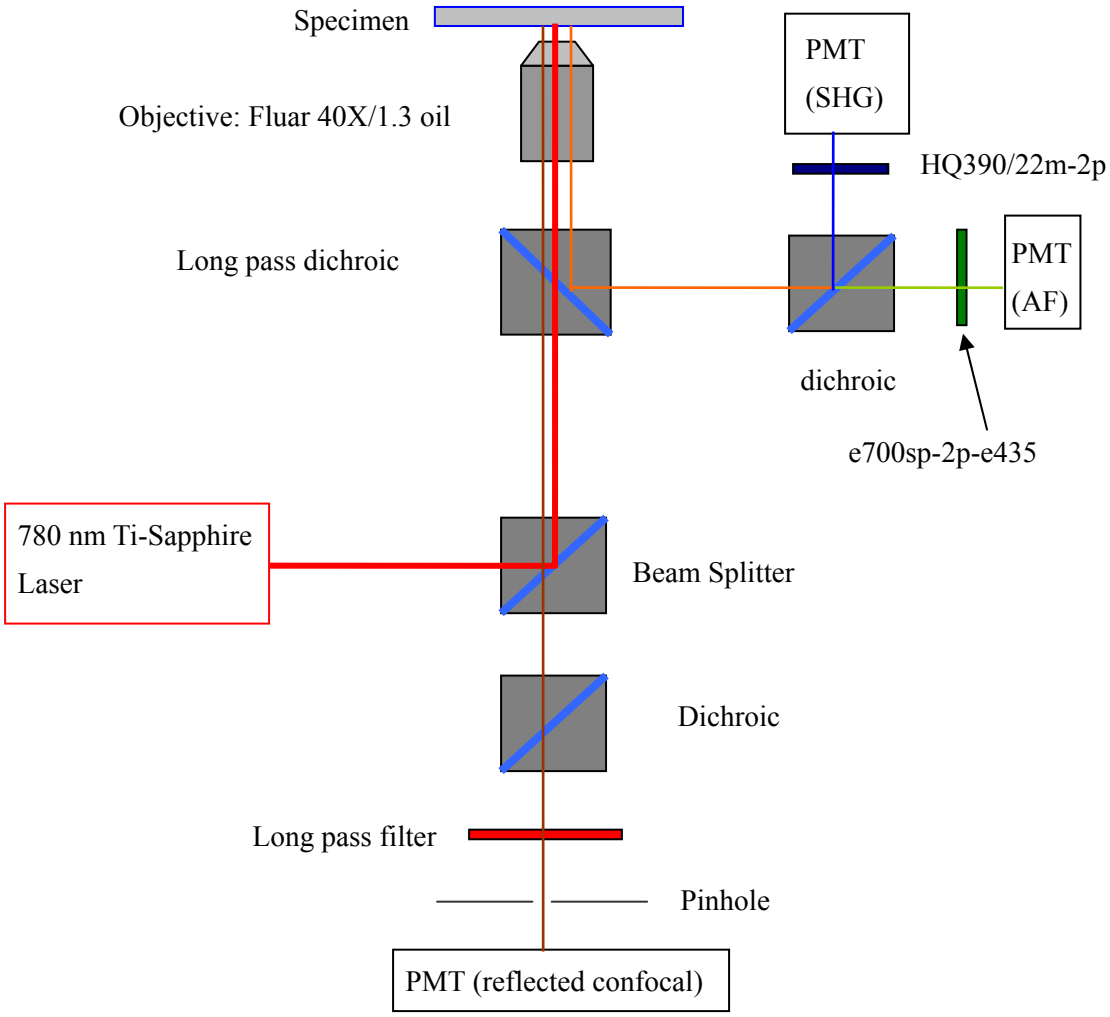

Fig. 1. The instrumental setup of the combined reflected confocal and multiphoton microscope. AF stands for autofluorescence signal and SHG stands for second harmonic generation signal.

3. Results

The reflected confocal signal (RC), autofluorescence signal (AF), second harmonic generation signal (SHG), and combination signal (Combined) are shown respectively in the image results. The images taken at different layers of skin are shown in Fig. 2 to Fig. 6. The morphology of the stratum corneum (horny layer) can be recognized by the reflected confocal signal (Fig. 2). For the stratum granulosum (granular layer), the autofluorescence signal comes mostly from the cell cytoplasm and the reflected confocal signal shows the cell membrane structures (Fig. 3). Our results show that the reflected confocal and multiphoton signals are complementary in characterizing the cell morphologies. When images are

taken deeper into the stratum spinosum (spinous layer) and stratum basale (basal layer), the cell borders are more clearly shown from reflected confocal signal and autofluorescence signal provides the cytoplasmic details (Figs. 4-5). The second harmonic generation signal is generated by collagen in the epidermis-dermis junction, but there is no reflected confocal signal at the same location (Fig. 6).

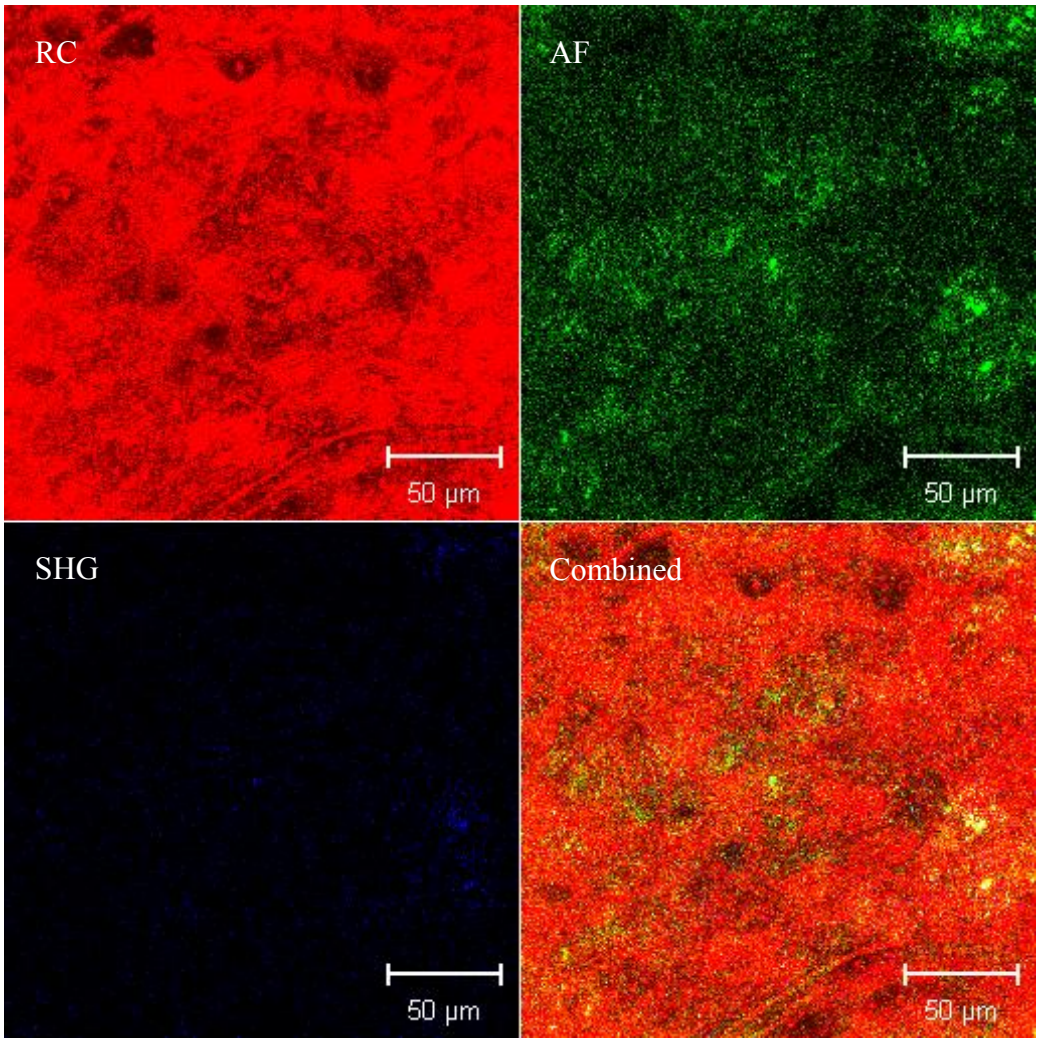

Fig 2. The images of the stratum corneum.

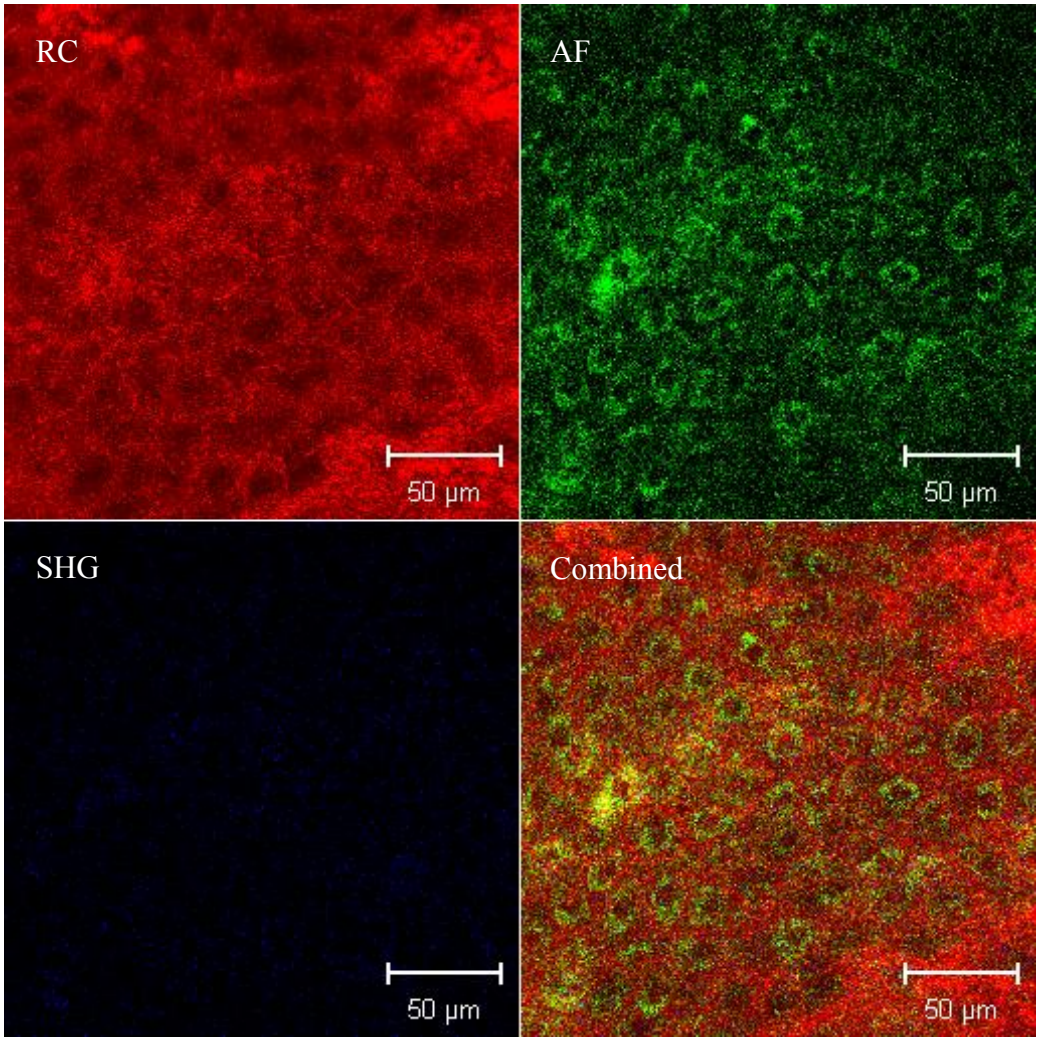

Fig 3. The images of the stratum granulosum.

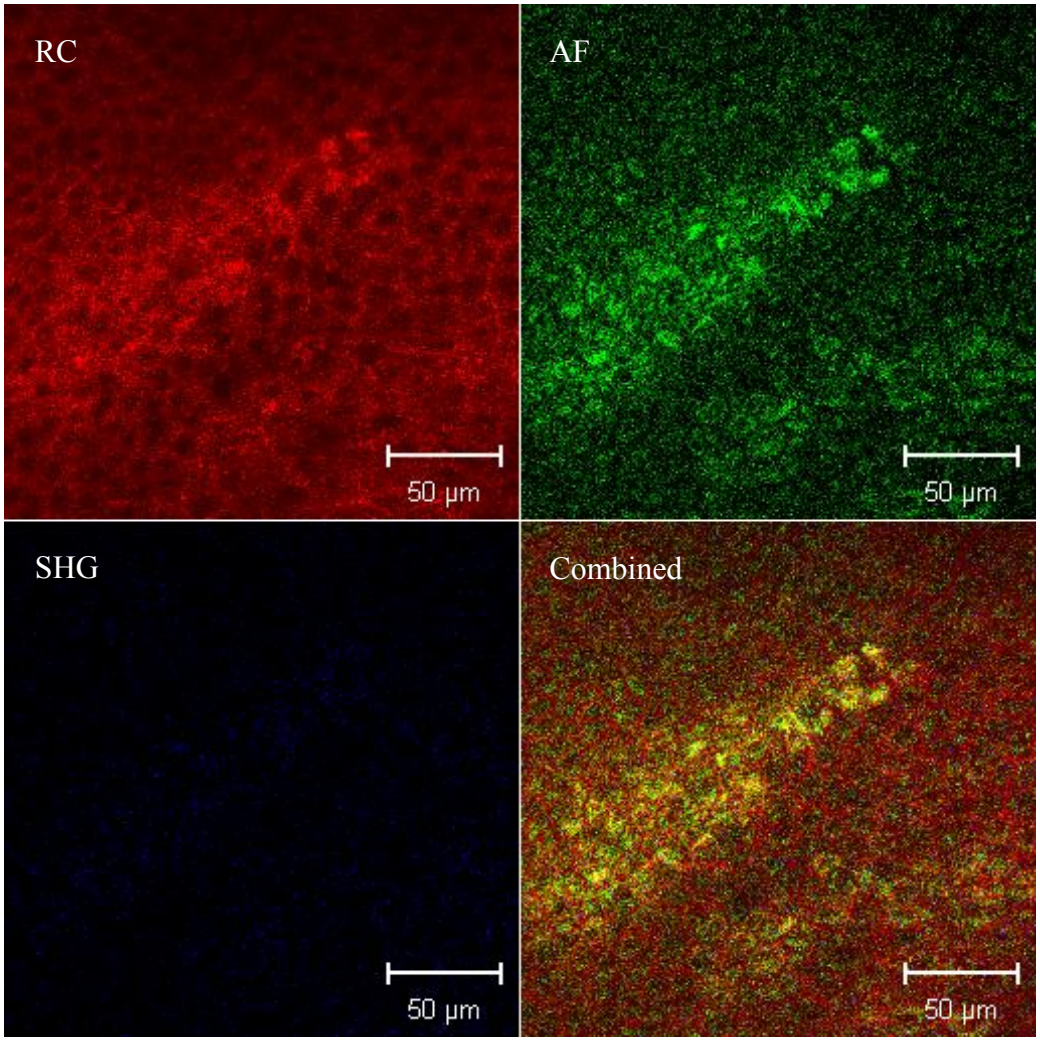

Fig 4. The images of the stratum spinosum.

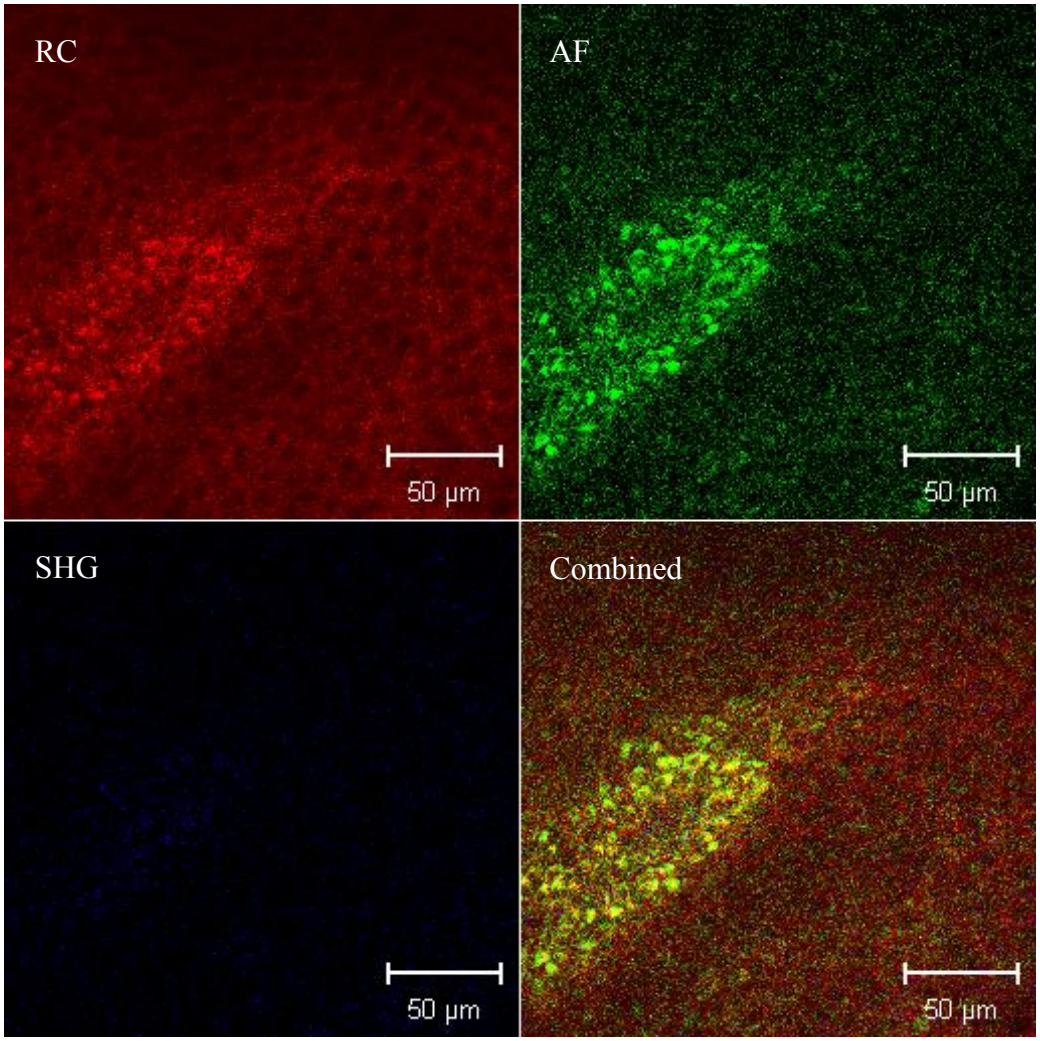

Fig 5. The images of stratum basale.

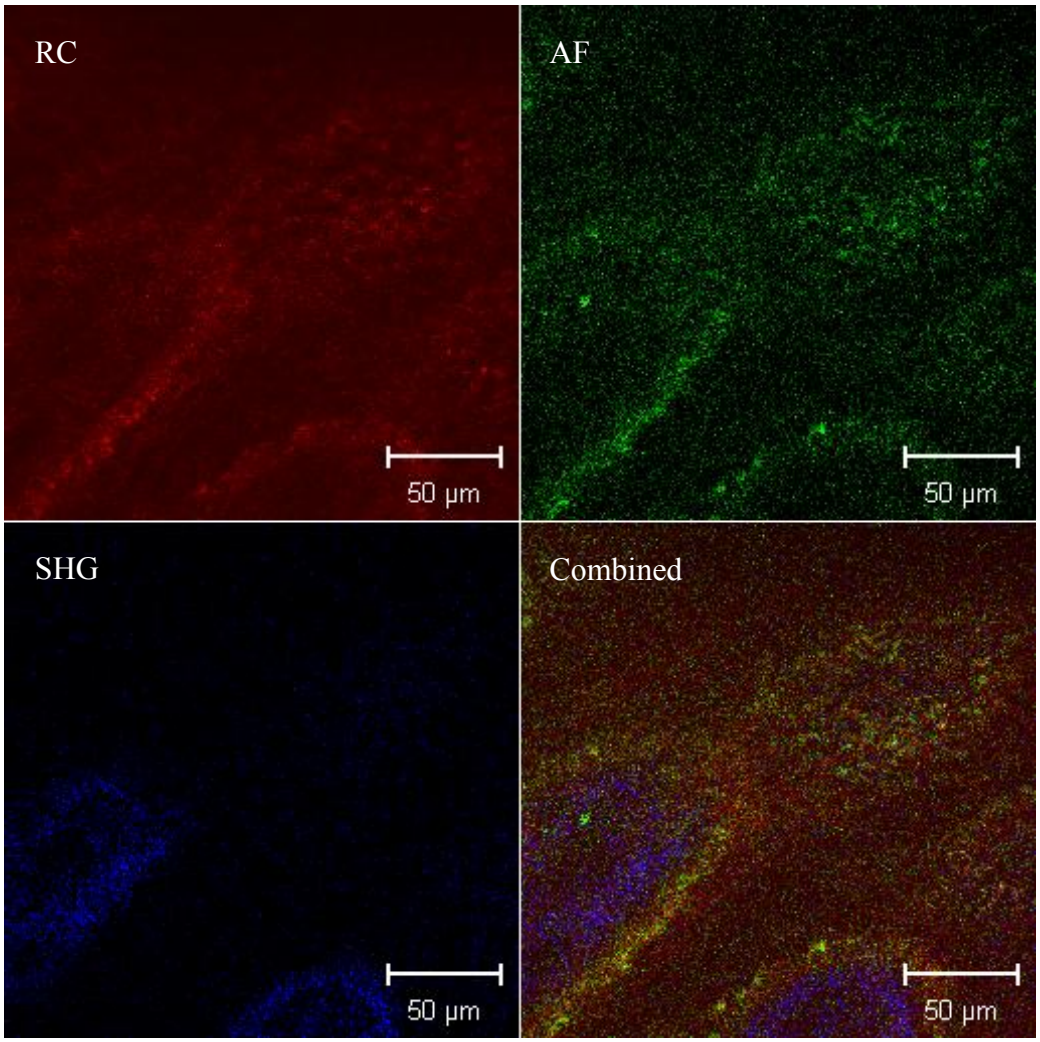

Fig 6. The images of the epidermis-dermis junction.

4. Conclusion

We have combined the reflected confocal and multiphoton microscopy to visualize the three-dimensional structures of skin. The reflected confocal signal is useful to help identify the cell borders while the multiphoton signal provides the cytoplasm morphology. The second harmonic generation signal is effective in collagen image. This multi-modality imaging technique has potential for clinical diagnosis in dermatology.

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