Raman spectroscopy of Chinese human skin *in vivo*

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A novel and compact near-infrared (NIR) Raman system is developed using 785-nm diode laser, volume-phase technology (VPT) holographic system, and NIR intensified charge-coupled device (CCD). Signal-to-noise ratio (SNR) and resolution are improved compared with ordinary acquisition method by a specially designed optical fiber detector and the spectrograph image aberration correction with a parabolic-line fiber array. In 1—5 s, Raman spectra of different parts of Chinese human skin are acquired. Autofluorescence is subtracted from the raw spectrum by polynomial fitting and skin Raman spectrum is then smoothed for further analysis.

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Raman spectroscopy is a rapid and accurate analytic probe which has been conducted to disease diagnosis and healthy treatment *in vitro*. But because of strong fluorescence signal and Rayleigh scattering interference of human living tissues, it is hard to acquire inherently weak Raman signal effectively and a long collection time is needed to reach good signal-to-noise ratio (SNR). Based on recent technical advances in compact and high performance near-infrared (NIR) diode laser and high-sensitivity charge-coupled device (CCD), some groups have studied several new NIR Raman systems and made some primary performances[1−6]. To our knowledge, so far there is no successful report on NIR Raman scattering of Chinese human tissues acquired *in vivo*. In this letter, a compact and rapid NIR Raman system is designed for clinical use, and the primary Raman spectra of Chinese human skin measured *in vivo* are reported.

The NIR Raman system consists of a 785-nm laser diode (LD), a transmissive holographic spectrograph, and a back-illuminated, deep-depletion NIR intensified CCD. For clinical applications, Raman measurements should be performed *in vivo* and real-time, preferably within seconds, and the system should display the results in time. Considering this basic requirement, we also specially designed a Raman fiber probe.

The schematic diagram of the system is shown in Fig. 1. The 785-nm excitation laser is focused on tissue surface with a 3.5-mm-diameter spot via a fiber, a collimating lens, a band-pass filter (785 ± 2.5 nm), and a focusing lens. All of the above optical components are then enclosed into a black drawtube having a 40° angle with human tissue. Raman photons scattered by human tissue are collected from the probe by a fiber bundle and fed into the spectrograph, and then are dispersed onto the CCD array detector by a volume-phase technology (VPT) holographic grating. A computer controls the CCD detector and the spectral data acquisition, processing, and display.

In order to reduce the interference of fluorescence and Rayleigh scattering, maximize the ability of Raman collection, and enhance the system SNR and resolution, a special fiber probe was designed, and the matching of the spectrograph's f-number to the numerical aperture (NA) of the fiber bundle was reached simultaneously. The collection probe is shown in Fig. 2. It can effectively reduce external interface and collect valid signals as well as facilitate the operation that using fiber bundle to collect Raman emissions from human skin. To best collect and transfer inherently Raman signals, as many fibers as allowed by the CCD detector height are packed into the bundle. In actual spectrograph, the entrance slit is limited, and image aberration occurrs when rays from different positions along the length of the slit are incident on a planar grating at different obliqueness. This causes significant distortion that can affect the measurement performance of the detector, leading to not only the decreases in spectral resolution and SNA,

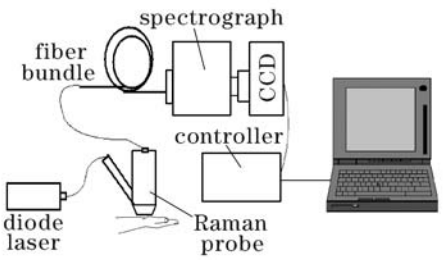

Fig. 1. Schematic diagram of the NIR Raman system.

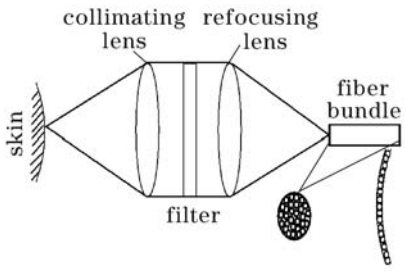

Fig. 2. Collection probe designed for the Raman system.

but also problem with wavelength calibration. To solve this question, fibers are arranged in circular shape at the connection to signal collection probe and a curved shape at the spectrograph entrance (as shown in Fig. 2). Adopting this method, the system SNR can be effectively improved.

The system covers a spectral range of 600—1800 cm^{-1} with a resolution of 4 cm^{-1}. Cyclohexane, acetone, and barium sulfate were measured after the installation was completed and the system was corrected and re-adjusted according to their standard spectra with an accuracy of ± 2 cm^{-1}.

Laser power which irradiated on human skin was measured first before the experiment to ensure that the exposure is not beyond American National Standards Institute (ANSI) standard. Human skins of arm, palm, upper arm, and ears were selected and put at the point of laser focus to obtain Raman signals with a collection time of 1—5 s, respectively. The raw *in vivo* Raman spectra of different locations of human being and the smoothed spectra after fluorescence subtraction are shown in Fig. 3. As can be seen from Fig. 3(a), in comparison with the strong fluorescence emission which is mostly originated from collagen, elastin, porphyrin, and other chromophores, Raman signals are some acuti-peaks overlaid fluorescence signals, but there are some Raman signals of human skin *in vivo* too weak so that they are overlaid by fluorescence and cannot be observed. To display Raman spectra more vividly and clearly, multi-degree polynomial is used to fit tissue fluorescence background and then the polynomial is subtracted from the measured spectrum to obtain Raman signals we need.

Raman peaks of Chinese human skin *in vivo* are assigned according to standard spectrum database and relative papers[7] as well as knowledge about skin biochemical composition, as shown in Table 1. Raman spectra of Chinese human skin show consistency in peak amount and position with other reported results[2,7], such as the υ(C=O) amide I band at 1654 cm^{-1}, the $\delta(CH_3)$ and $\delta(CH_2)$ scissoring mode at 1444 cm^{-1}, the $\delta(CH_2)$ wagging and twisting vibration at 1304 cm^{-1}, and the δ(NH) and υ(CN) amide III bands at 1270 cm^{-1}.

In the experiment, under the same conditions, the relative Raman intensity of the skin with deeper color is weaker than the whiter skin. And this can be explained as that the skin with deeper color has more melanin, which can absorb more NIR photons and transfer them into heat, while the NIR laser can penetrate whiter skin deeper to produce more Raman scattering.

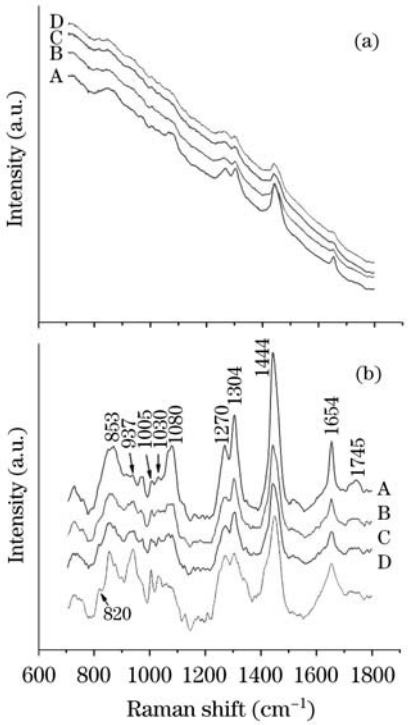

Fig. 3. (a) Raman spectra of Chinese human skin obtained *in vivo* and (b) the smoothed spectra after fluorescence subtraction. Curves A — D correspond to different locations of human being: A, ear; B, volar side of forearm; C, back surface of the forearm; D, palm of the hand.

Because of the diverse skin layer thicknesses and compositions, various physical states, as well as changeable environments human skin stays, different skin locations show different Raman spectra, even some subtle difference can be observed when comparing the same location of different individuals. In Fig. 3, under the same experimental conditions, ears show stronger Raman intensity than other parts of human skin. The ear skin is thin and more uniform, and then more Raman signals of dermis and subcutaneous tissues are acquired. So it is

Table 1. Raman Assignments of Chinese Human Skin *in vivo*

Raman Shift (cm^{-1})	Protein	Fat	Other Contributions
1745		υ(C=O)	
1654	υ(C=O) Amide I		
1444	$\delta(CH_2)$, $\delta(CH_3)$	$\delta(CH_2)$, Scissoring	
1304		$\delta(CH_2)$, Twisting, Wagging	
1270	υ(CN), δ(NH), Amide III		
1080		υ(CC), Skeletal	υ(CC), $\upsilon_5(PO_2^-)$
1030		υ(CC), Skeletal	Nucleic Acids
1005	υ(CC), Phenyl Ring		
937	υ(CC), Proline, Valine		
853	δ(CCH), Aromatic, Olefine		Polysaccaride
820	δ(CCH), Aliphatics		

possible to diagnose human beings physical states and healthy conditions according to statistical differences or rules which can be obtained if we have enough Raman spectra of healthy/pathological human skins.

In summary, using 785-nm LD laser, VPT holographic system, and NIR intensified CCD detector, a novel and compact NIR Raman system was developed to facilitate clinical NIR Raman detection. SNR and resolution were improved by a specially designed optical fiber probe and the spectrograph image aberration correction with a parabolic-line fiber array. Furthermore, in 1—5 s, Raman spectra of different parts of Chinese human skin were measured. Tissue autofluorescence was subtracted from the raw spectrum by polynomial fitting and skin Raman peaks are then assigned. The resolution and sensitivity of the system will be further improved and Raman spectra of individual skins and other tissue organs are carried on.

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