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Dual-phase stimulated Raman scattering microscopy for real-time two-color imaging: supplementary material

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This document provides supplementary information to "Dual-phase stimulated Raman scattering microscopy for real-time two-color imaging," http://dx.doi.org/10/1364/optica.4.000044. The auto-correlation and interference curves of the laser beams are presented before and after the pulses are chirped. A more detailed description of the working principle of the dual-phase SRS microscopy is supplied, and the cross-talk of the system is evaluated. In the last section, experimental materials and methods are provided.

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1. Supplement Figures

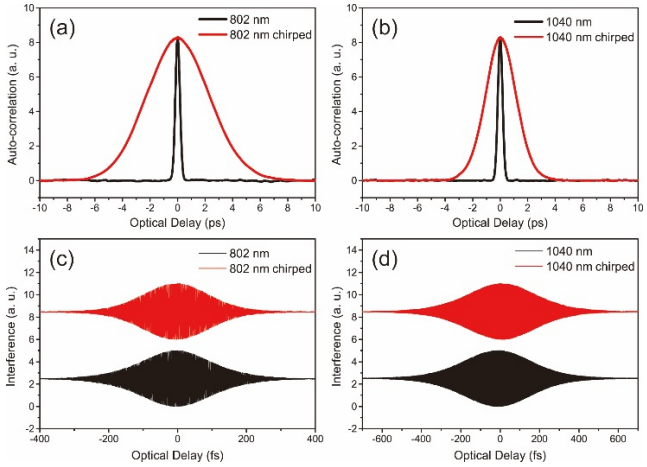

Fig. S1. Pulse duration and coherence time measurements of the pump and Stokes beam. (a) and (b) give the auto-correlation of the pump and Stokes pulses before and after they were chirped through NSF57 glass rods. (c) and (d) show the corresponding interferograms. While the pulse durations have been prolonged due to group velocity dispersion, the coherence times remain the same.

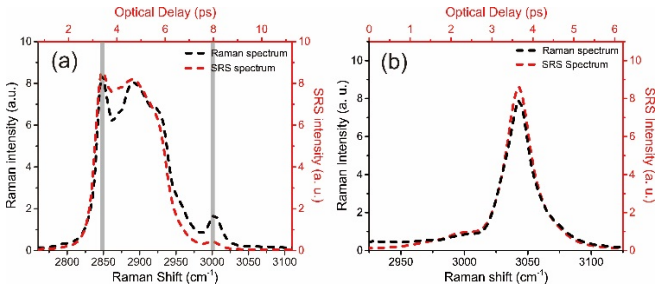

Fig. S2. Characterization of spectral focusing based SRS with spontaneous Raman spectra. Comparison between the two techniques on OA (a) and chloroform (in Phenol vol/vol=1:1) (b). Under linear chirp approximation, the estimated linear coefficient between Raman shifts and delay time was K=34.0 cm^{-1}/ps. And from the line shape analysis of the chloroform spectra, we estimated the spectral resolution of our SRS system to be ~ 23 cm^{-1}.

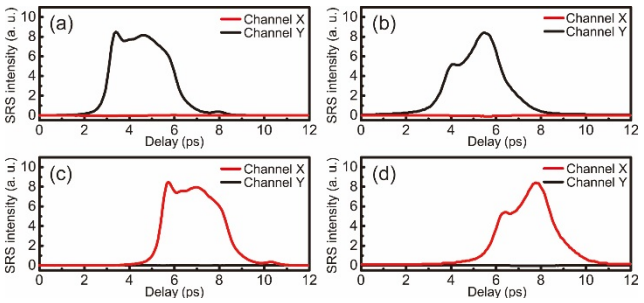

Fig. S3. Dual-phase SRS spectra of standard test chemicals. When S2 was blocked, the dual-channel spectra of oleic acid (a) and bovine serum albumin (b) were shown with vanishing signal in Y channel; and vice versa, when S1 was blocked, oleic acid (c) and bovine serum (d) albumin showed little signal in X channel.

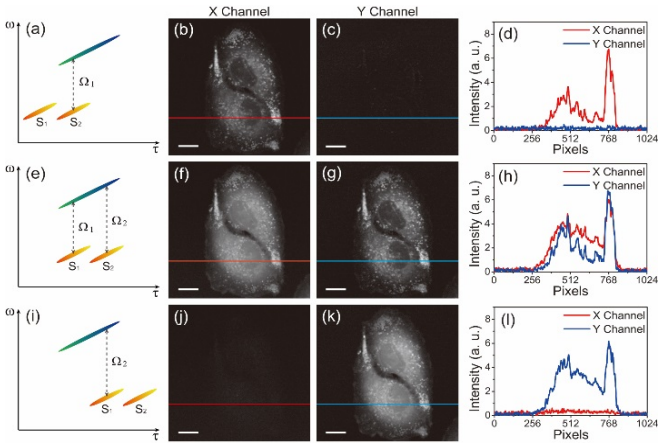

Fig. S4. Live cell imaging with different timing conditions. (a) S_1 was off-resonance (2770 cm^{-1}), and S_2 was set to probe 2848 cm^{-1}, corresponding images were shown in X (b) and Y (c) channels, and line-cut profile in (d), indicating a cross-talk of ~2%. (e-h) The two Stokes beams were set to detect 2848 cm^{-1} and 2926 cm^{-1} simultaneously. (i-l) S_1 was set to 2926 cm^{-1} and S_2 was set to probe 3115 cm^{-1}. Scale bar: 10 μm.

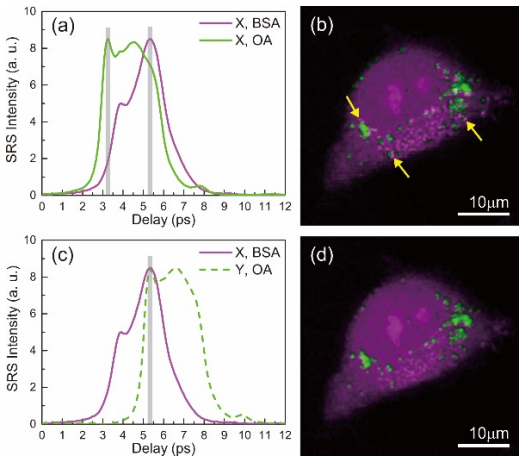

Fig. S5. Investigation of motion artifacts. (a) Sequential tuning of time delay with S_1 beam only to image 2848 cm^{-1} and 2926 cm^{-1}(gray bars). (b) Composite lipid (green) and protein (magenta) image of a HeLa cell appears artificial protein droplets (arrows) caused by moving lipid droplets. (c) Simultaneous imaging of 2848 cm^{-1} and 2926 cm^{-1} with dual-phase method. (d) Little motion artifacts were seen in the composite image.

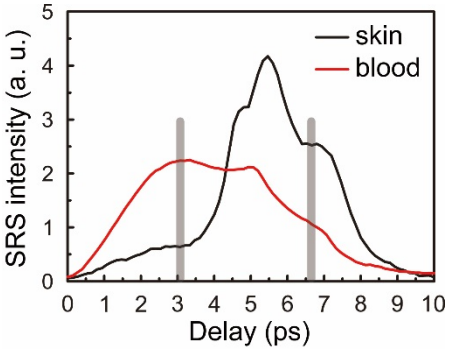

Fig. S6. Spectra of mice skin tissue and red blood cells. The spectrum of skin tissue represents mainly proteins, while that of the red blood cells mainly originates from the combination two-photon absorption of hemoglobin and SRS of protein. The pump beam was tuned to 807nm to slightly increase the hemoglobin signal. Gray bars mark the positions for real-time two color imaging. Simple linear algebra has been used to decompose the raw data into the hemoglobin and protein distributions [1, 2].

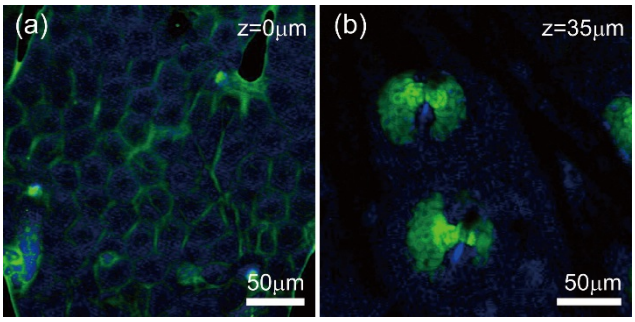

Fig. S7. *In vivo* two-color images of mice skin in epi mode. (a) stratum corneum, (b) sebaceous glandand.

2. Materials and Methods

A. Spontaneous Raman Spectrometer

The Raman spectra were acquired using a homebuilt Raman microspectrometer including a microscope (IX70; Olympus), a spectrometer (iHR320 and Symphony CCD; Horiba) and a Helium-Neon laser (1145P; JDSU). The average power on the sample was ~10 mW. The spectra were acquired by a custom Labview program and processed using LabSpec software.

B. Chemical Agents and Cell Culture

Oleic acid (OA) and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich. HeLa cells and A549 cells were maintained in DMEM medium (Gibco) supplemented with 10% (vol/vol) calf serum, 100 units/mL penicillin, 100 μg/mL streptomycin in a humidified standard incubator with a 5% (vol/vol) CO_2 atmosphere at 37 °C. The cells were imaged in phosphate buffered saline when they reached a moderate confluence.

C. Mouse Brain Sections and Live Animal Imaging

Mice were bred under standard husbandry conditions of Fudan University on a 12h light/dark cycle, and all experiments were performed in accordance with the Animal Care and Use Committee at Fudan University.

For ex vivo brain imaging, frozen and fixed sections were utilized. Mouse was perfused with PBS and 4% paraformaldehyde in phosphate buffered saline. Then the brains were fixed in 4% PFA overnight at 4 °C, and transferred sequentially to 15% and 30% sucrose after brain sunk to the bottom. Brains were embedded and frozen in O.C.T. compound (Tissue-Tek) and 40um serial sections were coronally prepared by frozen microtome (Leica, CM1950) and packaged between two glass slices when ready for imaging.

In vivo mouse skin imaging was carried out in epi-mode as previously did [1, 3]. A specially designed annular epi-detector was employed to detect the backscattered signal from the live target. Mouse was anaesthetized with its ear flipped over the head and covered by a thin cover slip, then imaged under the SRS microscope. All the mice were sacrificed after the measurements.

Zebrafish Embryos were obtained from natural spawning of wildtype (AB*) zebrafish line. Embryos were raised into 5dpf larvae in E3 medium with 1-Phenyl-2-Thiourea (PTU) at 28.5 °C. For imaging, the larvae were anesthetized in 0.003% tricaine, and were mounted in 4% methyl cellulose (Sigma M0512) between two spaced cover slides.

D. Imaging Parameters

Laser power of 37 mW (pump) and 50 mW (Stokes) at the sample were used for transmission mode imaging, and doubled for epi mode imaging. Pixel dwell time of 2 us and image size of 512x512 were applied throughout the experiment, except for in vivo imaging of the heart beat and blood stream, where 0.5 us pixel dwell time and 256x256 imaging size were used instead. Cell images were taken by a 60x objective (Olympus, UPLSAPO 60XWIR) and tissues image were taken by a 25x objective (Olympus, UPLSAPO 25XWMP2).

E. Measuring $\Delta T/T$

First we measured the SRL voltage in a reference sample (oleic acid) from the lock-in amplifier with 50 Ohm impedance, which represents ΔT; then we measured the transmitted pump power and its generated DC voltage from the photo-diode with 50 Ohm termination, which corresponded to T. In this way we can get $\Delta T/T$ of our reference sample. Finally, we calculated $\Delta T/T$ of other samples by scaling with the signal intensity and transmitted pump power. With the above laser parameter, our measured of OA $\Delta T/T$ is $\sim 8 \times 10^{-4}$, and one order of magnitude smaller in cell imaging.

References

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