Supplementary information

In vivo and in situ spectroscopic imaging by a handheld stimulated Raman microscope

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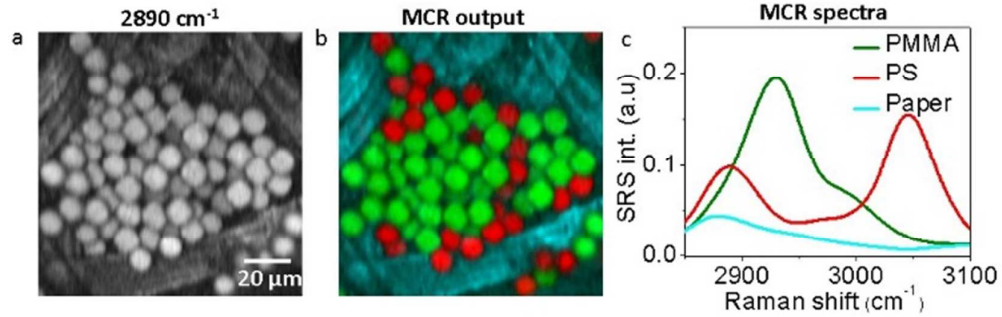
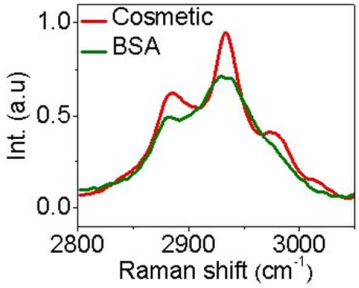
High speed imaging of microsphere mixture



Supplementary Figure 1: Spectroscopic imaging of PS/PMMA microsphere on a paper. (a) SRS image at 2890 cm^{-1}. (b) MCR concentration map and (c) output spectra.

To demonstrate real-time hyperspectral SRS imaging, we scanned a sample of mixed dried microspheres of PS and PMMA (Polyscience) on a piece of paper at the speed of 8 frames per second at 2890 cm^{-1} with 200x200 pixels (**Supplementary Figure 1a**). We first diluted 5 µm PS and PMMA with pure water by 100 times, respectively, and mixed two diluted solutions. Then a droplet of 5 µL mixture was placed on a paper. The spectroscopic images were acquired by sequentially tuning the optical delay in each spatial line and then 200 lines in one image. Total 200 spectra within each spatial line were acquired in 0.56 ms, and a spectroscopic image with 20 spectral points was completed within 3 seconds. The MCR output concentration maps of PS, PMMA and cellulose fibres of paper indicated their spatial distributions ((**Supplementary Figure 2b**), and the output spectra matched the spontaneous Raman data shown in **Figure 2.**

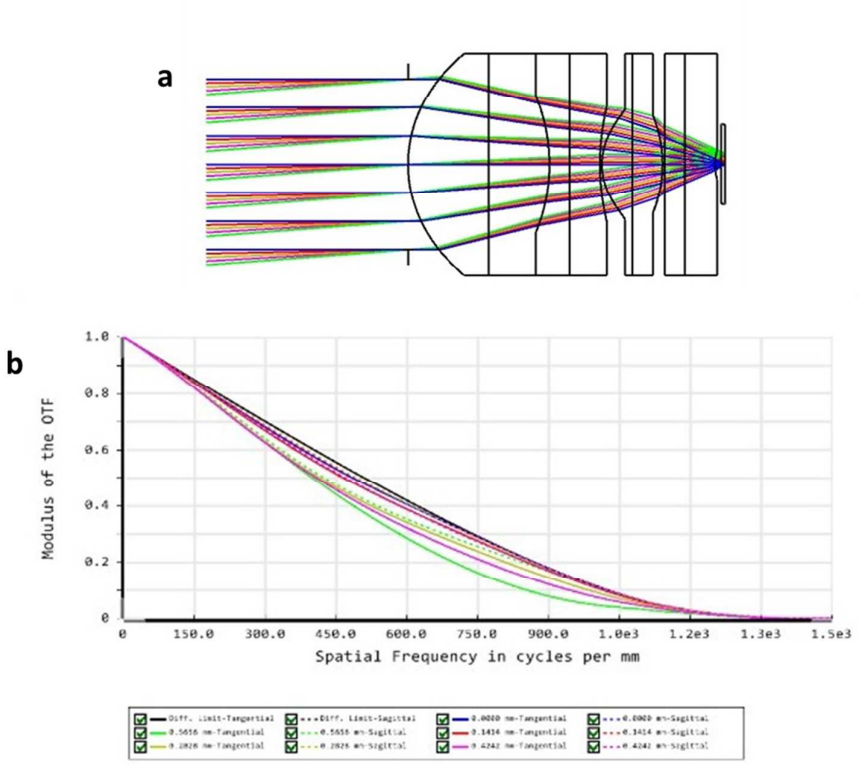
Spectral features of protein and commercial cosmetic



Supplementary Figure 2: Spontaneous Raman spectra of a commercially available cosmetic

To identify the spectral features of a commercially available cosmetic which is mostly composed of vitamin E and human skin which mostly exhibits protein signals, we acquired spontaneous Raman spectrum from the cosmetic (Raphe Pharma Labs) and BSA (Sigma Aldrich) **(Supplementary Figure 2)**. In CH vibrational region (2800-3000 cm^{-1}), cosmetic exhibits a broader spectral profile.

Homebuilt objective lens



Supplementary Figure 3: Simulation result of homebuilt objective lens. (a) Lens configuration of the objective lens. (b) Modulation transfer function of the objective lens

The lens configuration of the microscope objective is shown in **Supplementary Figure 3a**. The microscope objective has a NA of 0.5. Lens 1 and lens 3 are made from polymethyl methacrylate (PMMA), and Lens 2 and Lens 4 are made from OKP-4 HT. There are four aspheric surfaces in this design. **Supplementary Figure 3b** shows the corresponding modulation transfer function (MTF). **Fig. 7** shows the lens elements fabricated by diamond turning process and the assemble objective. We can clearly see the mechanical structures outside of lens clear apertures that serve as assembling and aligning purpose.

Supplementary video caption

Video 1: Images of living human skin at a speed of 8 frames per second in handheld setting