Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

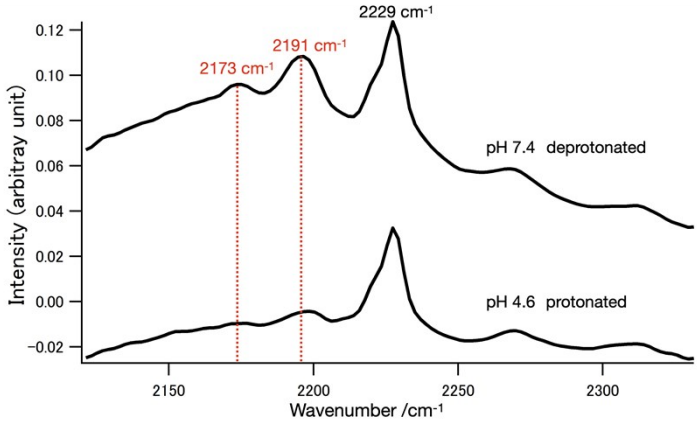

Fig. S1 Absorption spectrum of protonated and deprotonated FCCP measured at pH 7.4 and pH 4.6, respectively.

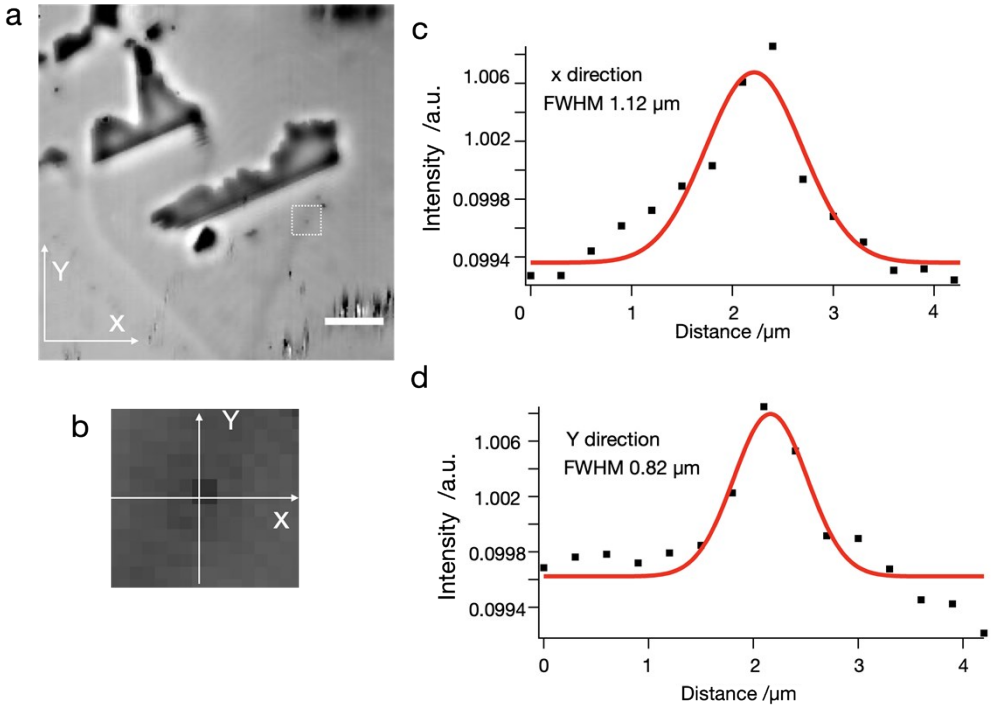

Fig. S2 FWHM of the FCCP particle in the bright-field image. (a) Bright-field image of FCCP particle. **(**b) Enlarged image of white box in (a). (c–d) Intensity profile of the bright-field image of FCCP particle in (b). FWHMs in x- and y-axes were 1.12 and 0.82 µm, respectively.

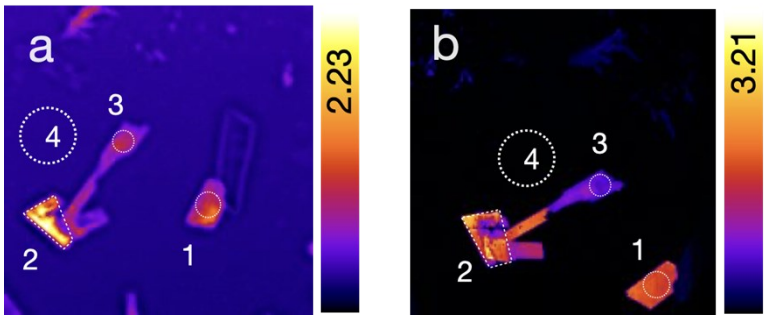

Fig. S3 Comparison of water background in IR-PTM and SRS. a) IR and b) SRS images at 2229 cm^{-1} (on-resonance). Position 4 is located in water. The ratios of FCCP signals and water signals in the region 1, 2, and 3 are 3.4, 2.8 and 2.7 in IR-PTM and 300, 273 and 124 in SRS microscopy. The FCCP-water ratios averaged in the three regions are 4 in IR-PTM and 232 in SRS microscopy.

SNR calculation for the experimental results using IR-PTM and SRS microscopy

To compare the SNR in two systems with different incident intensity, the photothermal signal S_{IR} needs to be normalized by the power of probe beam, the noise N_{IR} obtained from the photothermal image needs to be normalized by the shot noise, which can be written:

$$SNR_{IR} \approx \frac{S_{IR}}{P_{probe}/h\nu_{probe}} / \frac{N_{IR}}{\sqrt{P_{probe}/h\nu_{probe}}} = \frac{S_{IR}}{N_{IR}\sqrt{P_{probe}/h\nu_{probe}}} \quad (1)$$

where S_{IR} is the PT signal obtained from the chosen region in the PT image, N_{IR} is the standard deviation obtained from the image obtained under the experimental conditions with the IR beam off.

The SRS signal S_{SRS} also needs to be normalized by the power of Stokes beam, the noise N_{SRS} obtained from the SRS image needs to be normalized by the shot noise, which is written in equation (2)

$$SNR_{SRS} \approx \frac{S_{SRS}}{P_{pump}/h\nu_{pump}} / \frac{N_{SRS}}{\sqrt{P_{pump}/h\nu_{pump}}} = \frac{S_{SRS}}{N_{SRS}\sqrt{P_{pump}/h\nu_{pump}}} \quad (2)$$

where S_{SRS} is the SRS signal obtained from the chosen region in the SRS image, N_{SRS} is the standard deviation obtained from the image obtained under the experimental conditions with the Stokes beam off.

We also calculated the light to signal conversion efficiency (*LSC*), which was defined as equation (3)

$$LSC_{IR} \approx \frac{S_{IR}}{N_{IR}P_{IR}\sqrt{P_{probe}/h\nu_{probe}}} \quad (3)$$

While the *LSC* in SRS microscopy is represented as

$$LSC_{SRS} \approx \frac{S_{SRS}}{N_{SRS}P_{Stokes}\sqrt{P_{pump}/h\nu_{pump}}} \quad (4)$$

SNR models for IR-PTM and SRS microscopy

For photothermal microscopy, the signal is described as [1]

$$S_{IR} \approx \frac{1}{5\pi\omega_0} n \frac{\partial n}{\partial T} \frac{1}{C_p \lambda^2 \Omega} \frac{\sigma_{IR}}{A_{probe}} P_{IR} P_{probe} \Delta t \quad (5)$$

where ω_0 is the focal radius of probe beam, n is the refractive index of the medium, $\partial n/\partial T$ is the temperature dependence of refractive index, C_p is the heat capacity of per unit volume, σ is the absorption cross section, λ is the wavelength of the probe beam, P_{probe} and P_{IR} are the incident powers of probe and IR beams, respectively, A_{probe} is the detection area, and Δt is the integration time of the lock-in amplifier. The equation was slightly modified from [1] to consider the difference of the duty ratio of the system (1/10 in our system and 1/2 in the reference).The noise was assumed to be from shot noise-limited detection, as:

$$N_{IR} \propto \sqrt{\frac{P_{probe} \Delta t}{h\nu_{probe}}} \quad (6)$$

Therefore, the signal to noise ratio (SNR) is given by the following equation:

$$SNR_{IR} \approx \frac{1}{5\pi\omega_0 \lambda^2 \Omega} n \frac{\partial n}{\partial T} \frac{1}{C_p} \frac{\sigma_{IR}}{A_{probe}} P_{IR} \sqrt{\frac{P_{probe} \Delta t}{h\nu_{probe}}} \quad (7)$$

Assuming the numerical aperture of the objective lens used in the photothermal system is 0.49, and the wavelength of the probe beam is 532 nm, the focal radius of the probe beam is then:

$$\omega_0 = \frac{0.61\lambda}{NA} = \frac{0.61 \times 532}{0.49} = 662\ nm \quad (8)$$

The frequency of the probe beam is:

$$\nu_{probe} = \frac{c}{\lambda_{probe}} = \frac{3 \times 10^8}{532 \times 10^{-9}} = 5.6 \times 10^{14}\ s^{-1} \quad (9)$$

Considering the refractive index of water is $n = 1.3$, the temperature dependence of refractive index under 25 degrees C is 9×10^{-5} K^{-1}, the heating beam was modulated at 100 kHz, heat capacity per unit volume of water is $C_p = 4.2 \times 10^6 J/m^3K$, and the integration time of the lock-in amplifier is 10 μs,

$$SNR_{IR} \approx 5.0 \times 10^8 \frac{\sigma_{IR}}{A_{probe}} P_{IR} \sqrt{P_{probe}} \quad (10)$$

For stimulated Raman Scattering (SRS), the SRS signal is written as [2]

$$S_{SRS} \approx b_{SRS} \frac{Im(\chi^{(3)})}{A_{pump} f_{rep} \tau_{SRS}} P_{Stokes} P_{pump} \Delta t \tag{11}$$

where b_{SRS} is a constant in a certain experimental condition, $Im(\chi^{(3)})$ is the imaginary part of $\chi^{(3)}$, $\chi^{(3)}$ is the third-order nonlinear susceptibility of the material, which is a function of molecular number density, molecule orientation, scattering cross section and the third-order hyperpolarizability of each molecule, f_{rep} is the repetition rate, τ_{SRS} is the pulse width of both the pump and Stokes beam, A_{pump} is the pump-beam waist as the detection area, P_{Stokes} is the incident power of the Stokes beam, P_{probe} is the incident power of the pump beam.

The shot noise is assumed to be the same as that in the photothermal system, which is written as equation (12),

$$N_{SRS} \propto \sqrt{\frac{P_{pump} \Delta t}{h \nu_{pump}}} \tag{12}$$

SNR of the SRS system is represented as the following:

$$SNR_{SRS} \approx b_{SRS} \frac{Im(\chi^{(3)})}{A_{pump} f_{rep} \tau_{SRS}} P_{Stokes} \sqrt{\frac{P_{pump} \Delta t}{h \nu_{pump}}} \tag{13}$$

Here, b_{SRS} is given as [3]:

$$b_{SRS} = \frac{3\ \pi z}{n_s n_p \varepsilon_0 c \lambda_p} \tag{14}$$

where the refractive index of the sample n_s and n_p are assumed to be $n_s = n_p = 1.3$, the permittivity is $8.85 \times 10^{-12}\ F/m$, the wavelength of the pump beam is $\lambda_p = 840\ nm$, and the focal length z is 1 μm. Therefore,

$$b_{SRS} = 2.4 \times 10^3 \tag{15}$$

while the frequency of the probe beam is:

$$\nu_{pump} = \frac{c}{\lambda_{pump}} = \frac{3 \times 10^8}{840\ \times 10^{-9}} = 3.6 \times 10^{14}\ s^{-1} \tag{16}$$

Considering the modulation frequency of the Stokes beam is 80 MHz, the pulse width τ_{SRS} of the pump and Stokes beam is 2 ps, the integration time Δt of the lock-in amplifier is 10 μs, the SNR of SRS system becomes:

$$SNR_{SRS} \approx 9.8 \times 10^{13} \frac{Im(\chi^{(3)})}{A_{pump}} P_{Stokes} \sqrt{P_{pump}} \tag{17}$$

Furthermore, $Im(\chi^{(3)})$ can be given as [4]:

$$Im(\chi^{(3)}) = \frac{2\ \pi c^4}{3 h \omega_s^4 \Gamma} \sigma_{sr} \Delta \tag{18}$$

where h is the Plank constant, ω_S is angular frequency of the Stokes beam, Γ is the half of the full width of half maximum of Raman line which is assuming 6 cm^{-1}, σ_{sr} is molecule cross section of Raman scattering, Δ is difference population level between ground state and vibrational state ($\Delta = 1$ when all molecules are in the ground state). Therefore, equation (18) becomes:

$$Im(\chi^{(3)}) = 3.8 \times 10^{3}\sigma_{sr} \tag{19}$$

Taking equation (19) to replace $Im(\chi^{(3)})$ in equation (17), SNR can be rewritten as:

$$SNR_{SRS} \approx 3.7 \times 10^{17} \frac{\sigma_{sr}}{A_{pump}} P_{Stokes}\sqrt{P_{pump}} \tag{20}$$

For further calculations, the detection area in the photothermal system is assumed to be the spot size of the probe beam, which is $1.37 \times 10^{-8}\ cm^2$, with a typical absorption cross-section of $\sigma_{IR} = 10^{-18}\ cm^2$ [5]. The SNR in the photothermal system then becomes:

$$SNR_{IR} = 5 \times 10^{8} \frac{10^{-18}}{1.37 \times 10^{-8}} P_{IR}\sqrt{P_{probe}} = 3.6 \times 10^{-2} P_{IR}\sqrt{P_{probe}} \tag{21}$$

While in the SRS case, the detection area is assumed as $5.1 \times 10^{-9}\ cm^2$, and with the scattering cross section of $\sigma_{sr} = 10^{-29}\ cm^2$ [2], the SNR in the SRS system becomes:

$$SNR_{SRS} \approx 3.7 \times 10^{17} \frac{10^{-29}}{5.1 \times 10^{-9}} P_{Stokes}\sqrt{P_{pump}} = 7.3 \times 10^{-4} P_{Stokes}\sqrt{P_{pump}} \tag{22}$$

From our quantitative calculations in equation (21) and (22), it is obvious that the SNR of the IR-PTM system is about 50 times higher than that of the SRS system when compared on the basis of the same power input.

Reference

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2) Z. Chi, DL. Zhang and Ji-Xin Cheng. *Annu. Rev. Biomed. Eng.,* 2015, **17**, 415-445.
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4) G. M. Mikheev, D.G. Kaluzhnyi, A.V.Popoov and T.N. Mogileva. *Tech. Phys.,* 2005, **50.2**, 220-226.

5) J. J. Harrison, N. D.C. Allen and P. F. Bernath. *J. Quant. Spectrosc. Rad. Trans*., 2010, **111**, 357–363.