Supplementary Materials for

Stimulated Raman scattering tomography for rapid 3D chemical imaging of cells and tissue

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S1. Real-time monitoring of 3D Brownian motion of 4.5 μm polystyrene (PS) beads (Raman shift at 2885 cm^{-1}(CH_2 asymmetric stretching)) in water by PM-SRST

Fig. S1 (a) shows an example of the six phase patterns used for real-time monitoring of 3D Brownian motion of PS beads in water, which are pre-loaded onto the cache of SLM for PM-SRST imaging. The phase patterns are displayed following a sequence of (i) to (vi) as shown in **Fig. S1 (a)**, which are synchronized by SRS image acquisition. **Fig. S1 (b)** shows the snapshot 3D images of PS beads distributed in water versus time captured by PM-SRST.

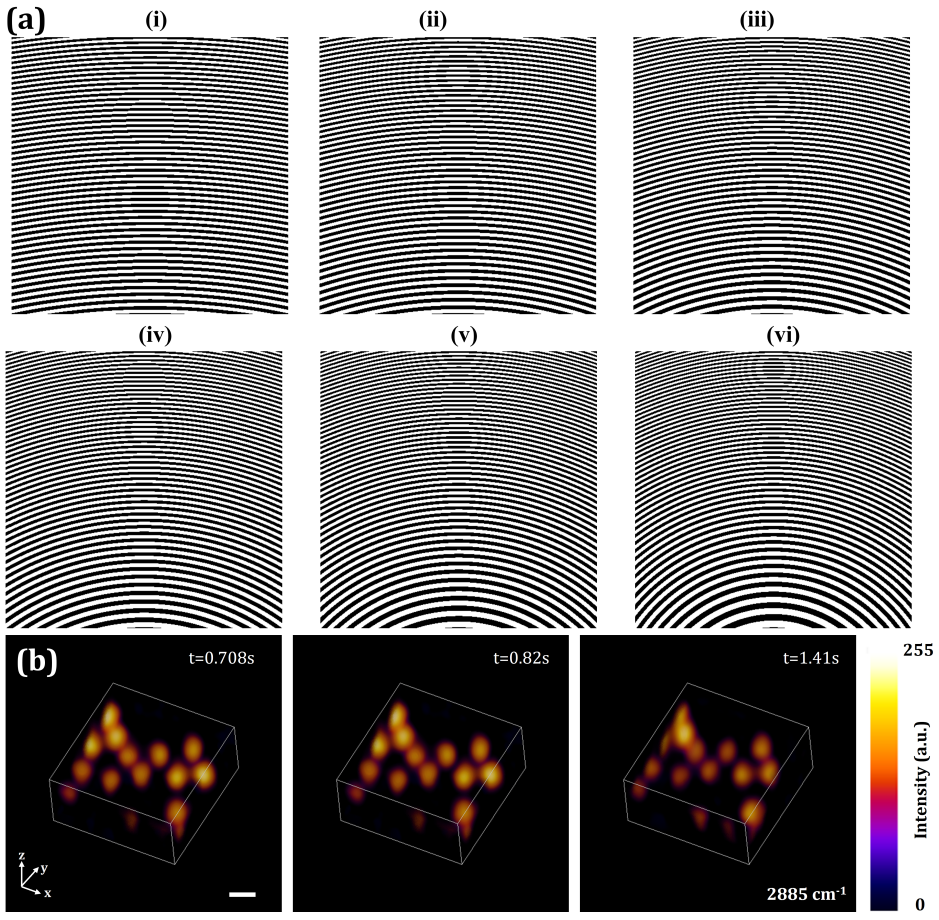


Fig. S1 (a) The six phase patterns generated ((i) to (vi)) corresponding to different depths are used for real-time monitoring of 3D Brownian motion of PS beads in water. (b) Snapshot SRS images (2885 cm^{-1} of CH_2 asymmetric stretching) of 3D Brownian motion of PS beads in water at different time by PM-SRST. Image volume: 23.08 μm × 23.08 μm × 20 μm. 64 × 64 pixels for 2D scanning; axial step size of 4 μm. Total 6 depths and 0.118 s interval are used for acquiring each 3D volume (8.5 Hz). Scalebar: 5 μm.

S2. Comparison of the laser intensity between the Gaussian Stokes spot and the Bessel Stokes beam by using two-photon fluorescence (TPF) imaging of 500 nm fluorescence beads.

Figs. S2 (a-b) show the comparison of x-y images of a 500 nm fluorescence bead obtained by using the Gaussian Stokes spot and Bessel Stokes beam (laser wavelength of 1041 nm) in TPF imaging. **Fig. S2 (c)** shows the estimated full width at half maximum (FWHMs) of the beads in **(a)** and **(b)** along the dashed line, in which the corresponding diameter D_G of 0.8 μm for Gaussian Stokes spot (NA~0.54) and diameter D_B of 0.7 μm for Bessel Stokes beam (NA~0.5) are obtained. **Figs. S2 (d)** and **(e)** show the comparison of x-z images of a 500 nm fluorescence bead corresponding to **(a)** and **(b)**, in which the length (L_G=4.4 μm, FWHM) of Gaussian Stokes spot and (L_B= 111.4 μm, FWHM) of Bessel Stokes beam are obtained, respectively. Therefore, the volume of the needle Bessel beam is ~19.4-fold (i.e., $\frac{V_B}{V_G} = \frac{L_B * \pi(\frac{D_B}{2})^2}{L_G * \pi(\frac{D_G}{2})^2} = \frac{111.4*(0.7)^2}{4.4*(0.8)^2}$, V_B and V_G represent the volumes of the Bessel beam and Gaussian spot, respectively) larger than that of Gaussian spot.

Fig. S2 (f) shows the comparison of axial intensity distributions of fluorescence beads excited by a tightly focused Gaussian Stokes spot (laser power of 1.4 mW on the bead) and a Bessel Stokes beam (laser power of 19 mW on the bead) in TPF imaging. The maximum TPF intensity of fluorescence beads excited by Gaussian spot is ~1.4-fold higher than that of the Bessel beam. Hence, assuming that the same laser power used for Stokes beam in SRS imaging, the tightly focused Gaussian Stokes beam used in PM-SRST gives a ~16-fold ($\frac{19\ mW}{1.4\ mW} \times \sqrt{1.4}$; $I_{TPF} \propto I_{Stokes}^2$) stronger in local laser intensity compared to the Bessel Stokes beam used in OBT-SRST.

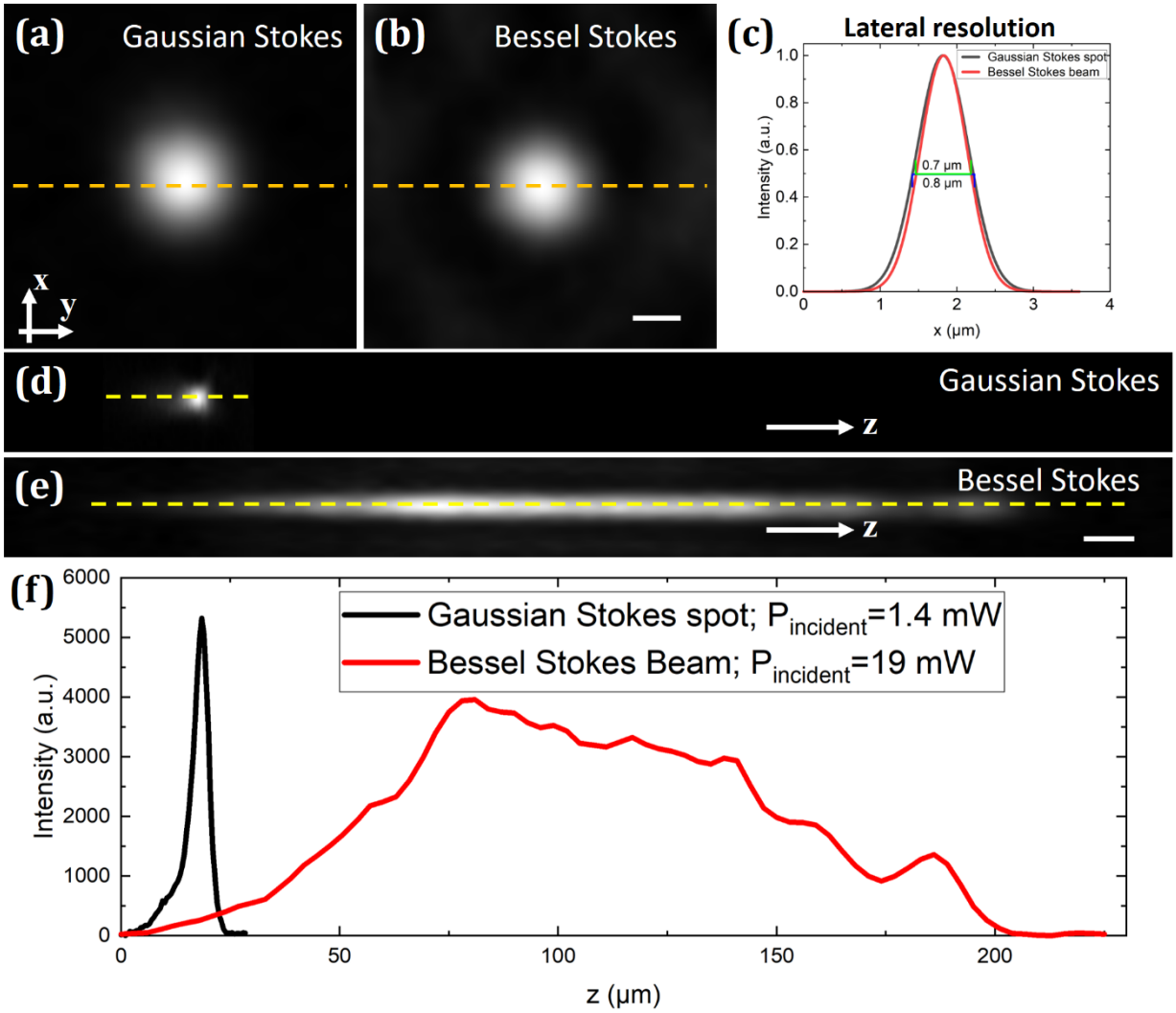


Fig. S2 (a-b) x-y images of a 500 nm fluorescence bead captured by TPF imaging using Gaussian Stokes spot and Bessel Stokes beam, respectively. Scalebar: 500 nm. (c) The TPF intensity distribution along the dashed line in (a) and (b) with the estimated FWHMs for Gaussian Stokes spot (0.8 μm) and Bessel Stokes beam (0.7 μm), respectively. (d-e) x-z images of the 500 nm fluorescence bead corresponding to (a) and (b), respectively. Scalebar: 10 μm. (f) The TPF intensity distribution along the dashed line in (d-e) with the estimated FWHMs for Gaussian Stokes spot (4.4 μm) and Bessel Stokes beam (111.4 μm). 256 × 256 pixels for beads imaging. Average powers of 1.4 mW for Gaussian Stokes beam and 19 mW for Bessel Stokes beam on the beads.

Videos for supporting content

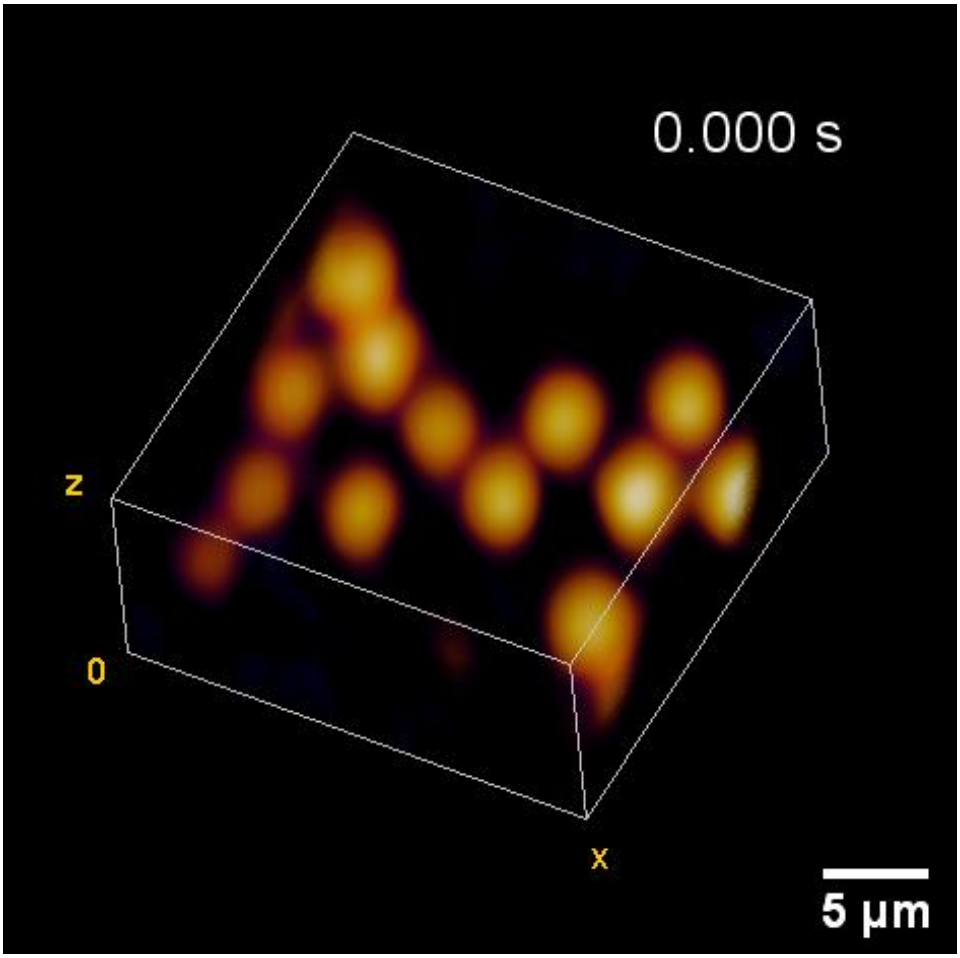
Video 1: Monitoring of 3D Brownian motion of PS beads (3D SRS at 2885 cm^{-1} of CH_2 asymmetric stretching) measured by PM-SRST. 64 × 64 pixels for 2D scanning, axial step size of 4 μm. Total 6 depths and 0.118 s acquisition time for capturing one 3D volume (8.5 Hz). (MP4, 630 KB)

Video 2: Diffusion and uptake processes (SRS at 2530cm^{-1} of O-D chemical bond) of D_2O into the plant root monitored by using PM-SRST. 256 × 256 pixels for 2D scanning, axial step size of 4 μm. Total 6 depths and 1.46 s acquisition time for obtaining one 3D volume. (MP4, 88 KB)

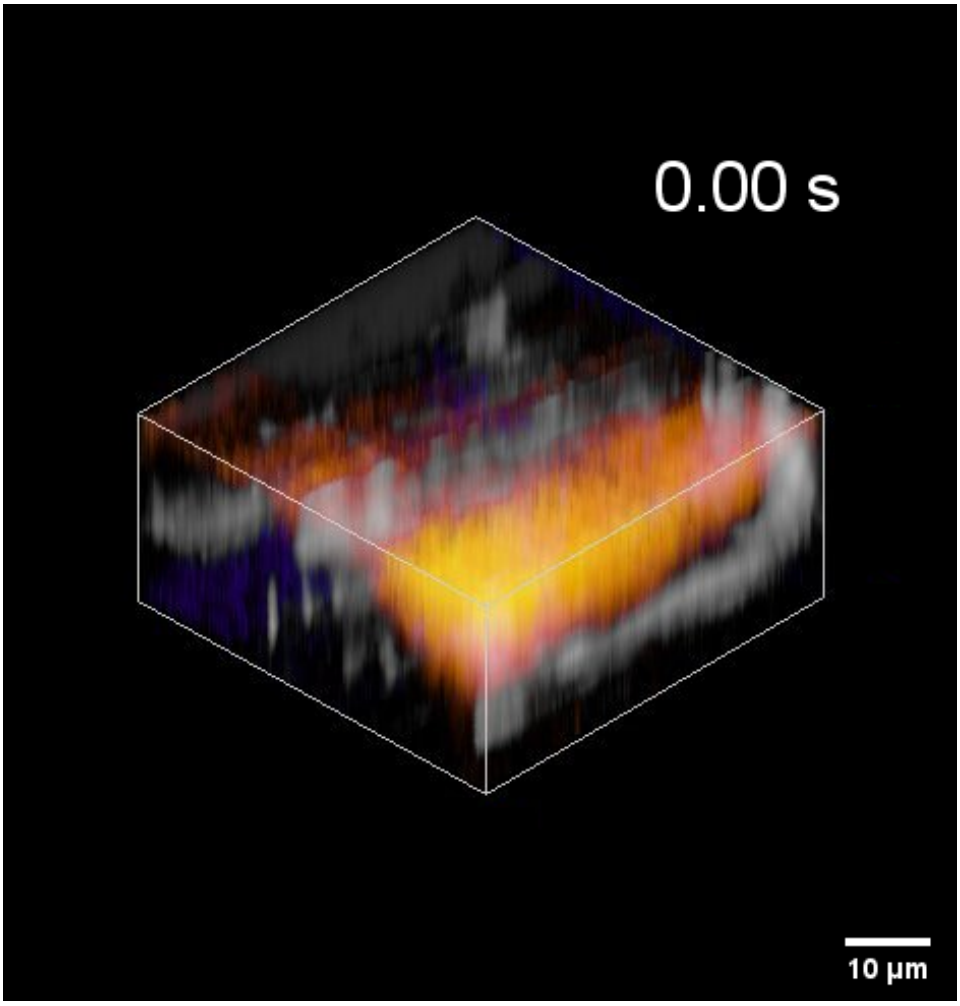
Video 3: Biochemical variations (SRS at 2935 cm^{-1} of CH_3 stretching of lipids and proteins) of MCF-7 cells subjected to acetic acid treatment by using PM-SRST. 256 × 256 pixels for 2D scanning, axial step size of 2.5 μm. Total 7 depths and 1.67 s time for acquiring one 3D volume. (MP4, 168 KB)

Video 4: Biochemical variations (3D SRS at 2845 cm^{-1} of CH_2 symmetric stretching of lipids) of MCF-7 cells subjected to acetic acid by PM-SRST. 256 × 256 pixels for 2D scanning, axial step size of 2.5 μm. Total 4 depths and 1.29 s time for acquiring one 3D volume. (MP4, 220 KB)

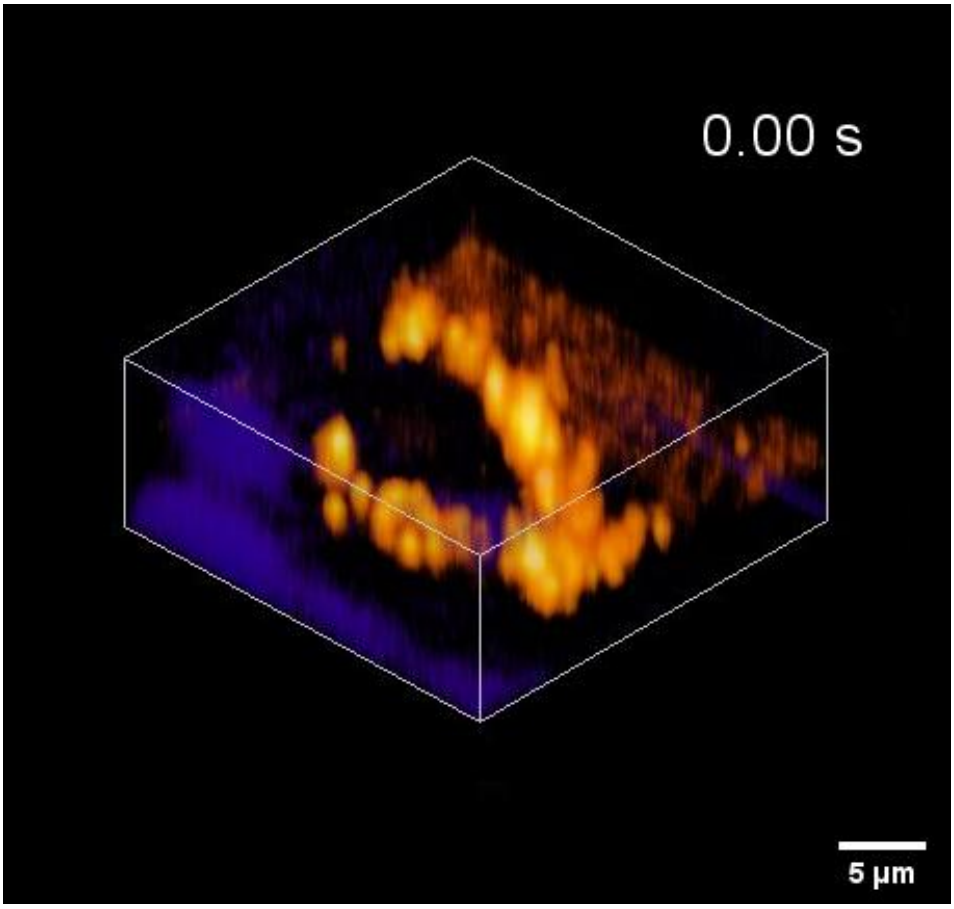
Video 1



Video 2



Video 3



Video 4