

Supporting Information for

Plasmonic Electronic Raman Scattering as Internal Standard for Spatial and Temporal Calibration in Quantitative Surface-Enhanced Raman Spectroscopy

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Theoretical analysis of SERS calibration by ERS signals

Figure 1A-left shows an energy-diagram illustration of the ERS process in plasmonic nanostructures. For Au at room temperature, most electronic states in sp -bands are occupied below the Fermi energy (E_F) following the Fermi-Dirac distribution.¹ In the ERS process, an electron in the conduction band close to the Fermi level can be optically excited to a virtual state and instantaneously relax to another state in the conduction band with slightly different energy and momentum following the E - k dispersion relation of sp -bands.²⁻⁴ Using a near-infrared (NIR) laser excitation (785 nm) with photon energy far below the interband transition energy of Au, one can avoid the interband transitions induced photoluminescence in the SERS background. The plasmonic ERS intensity is proportional to the density of electron-hole pairs, n_{e-h} , in metal nanostructures²⁻⁴ expressed as $n_{e-h}(\Delta\omega_e) = \left| \exp\left(-\frac{\hbar\Delta\omega_e}{k_B T}\right) - 1 \right|^{-1}$ where $\Delta\omega_e$ is the Stokes-shifted frequency for the ERS process, \hbar is the Planck's constant, k_B is the Boltzmann constant, and T is the temperature. Therefore, the ERS intensity exponentially increases when $\Delta\omega_e$ approaches zero. Under the continuous-wave (CW) laser excitation with low powers (< 1 mW), the conduction-band electrons and lattice phonons in metal nanostructures can reach thermal equilibrium to have nearly the same temperature through fast relaxation of photo-excited hot electrons. At the low-wavenumber range of energy shifts, ERS signals can dominate the SERS emission background by NIR laser excitation in Au nanostructures since intraband transition photoluminescence additionally requires significant momentum Δk to match the sp -band electron dispersion (Figure 1A-left).^{3,4} Compelling evidence has recently confirmed this case under the right condition, although it is still insufficient to explain light emission from plasmonic nanostructures under ultrafast pulsed laser excitation.⁵⁻⁷ In the ultrafast regime, where the pulse-width of lasers is compressed to an ultrashort timescale such as pico- or femtosecond comparable

or shorter than the electron-phonon scattering lifetime, both theoretical and experimental studies show that the energy of the photoexcited hot electrons are accumulated in the temporal domain, and thus the electrons form a Fermi-Dirac distribution with a much higher temperature (thousands of K) than the lattice.^{5, 7-10}

For the case of excitation with NIR CW, by using a long-pass filter to block the laser line (Rayleigh scattering), the filtered ERS background continuum exhibits an ERS pseudo-peak with signal intensity $I_{\text{ERS}}(\omega_o - \Delta\omega_e)$ in the measured Raman spectra (Figure 1A-right), where ω_o is the laser excitation frequency. Similar to the SERS mechanism for molecular vibrational modes, ERS signals also follow the fourth power of local field enhancement by plasmonic enhancement of both excitation and inelastic electronic scattering transitions.^{3,4} Therefore, surface plasmon enhanced ERS signal intensity can be expressed as:

$$I_{\text{ERS}}(\omega_o - \Delta\omega_e) = g_M(\omega_o)^2 \cdot g_M(\omega_o - \Delta\omega_e)^2 \cdot \sigma_{\text{ERS}}(\omega_o, \Delta\omega_e) \cdot |n_{e-h}(\Delta\omega_e) + 1| \cdot A \cdot t_M \cdot I_o(\omega_o)$$

(S1)

where g_M is the average field-enhancement factor in the metal side of the hotspot, σ_{ERS} is the effective cross-section for the ERS process, A is the metal surface area of a plasmonic nanostructure unit, t_M is the average field penetration depth in metal and I_o is the intensity of incident laser. In terms of g_M , although integral over the entire hotspot should be conducted to acquire precise enhancement factor, we simplify g_M as the averaged enhancement factor since the electric field inside of the metal exponentially decays along the z -direction.^{11,12} σ_{ERS} depends on the material property of the metal and consistent with the bulk material.¹³ n_{e-h} is expressed as $|n_{e-h}(\Delta\omega_e) + 1|$ considering the overall thermodynamic factor for the Stokes-shifted ERS process.^{2,14} While ERS signals show a continuous spectral feature because of the continuous

electronic *sp*-bands of the metal, molecular Raman scattering (MRS) signals carry many distinct narrow peaks in the measured spectra due to discrete inelastic energy shifts associated with discrete vibrational modes of a molecule (Figure 1B). Following the fourth power of local field enhancement, surface plasmon enhanced MRS signal intensity for a molecule placed in the plasmonic hotspot can be expressed as:

$$I_{\text{MRS}}(\omega_o - \Delta\omega_m) = g_I(\omega_o)^2 \cdot g_I(\omega_o - \Delta\omega_m)^2 \cdot \sigma_{\text{MRS}}(\omega_o, \Delta\omega_m) \cdot N \cdot r \cdot A \cdot t_I \cdot I_o(\omega_o) \quad (\text{S2})$$

where g_I is the field-enhancement factor in the insulator side of the hotspot, σ_{MRS} is the cross-section for the MRS process, N is the concentration of the analyte molecules in hotspots, r ($0 \leq r \leq 1$) is the effective coefficient related to the orientation of transition dipole moment for a specific vibrational mode of analyte molecules at hotspots, and t_I is the average field penetration depth in the analyte-accessible environment. As both ERS and MRS signals originate from the same hotspots, the factors including A and $I_o(\omega_o)$ are no longer relevant. Therefore, we can express the ratio between I_{MRS} and I_{ERS} from the same hotspots as:

$$\frac{I_{\text{MRS}}}{I_{\text{ERS}}} = \frac{g_I(\omega_o)^2 \cdot g_I(\omega_o - \Delta\omega_m)^2 \cdot \sigma_{\text{MRS}}(\omega_o, \Delta\omega_m) \cdot t_I \cdot N \cdot r}{g_M(\omega_o)^2 \cdot g_M(\omega_o - \Delta\omega_e)^2 \cdot \sigma_{\text{ERS}}(\omega_o, \Delta\omega_e) \cdot t_M \cdot |n_e - n_h(\Delta\omega_e) + 1|} \quad (\text{S3})$$

Since the electromagnetic boundary condition relates the dominant perpendicular electric fields at the metal-insulator interface at plasmonic hotspots, one can convert the terms of local field enhancement factors (g_M and g_I) into materials permittivity values at the metal-insulator interface based on the boundary condition. More specifically, we assume that the absorbed photons only induce a momentum change along with the z -direction, and thus only the z -component of the electric field contributes to the enhancement of the light absorption and emission with the continuous condition on the interface. Considering $g_I(\omega_o) \approx g_I(\omega_o - \Delta\omega_m)$ and $g_M(\omega_o) \approx$

$g_M(\omega_o - \Delta\omega_e)$ based on approximations of $\omega_o \approx \omega_o - \Delta\omega_m \gg \Delta\omega_m$ and $\omega_o \approx \omega_o - \Delta\omega_e \gg \Delta\omega_e$, the relation between near-field enhancement factors, g_I and g_M , can be expressed as:

$$\frac{g_I}{g_M} = \frac{E_{I-z}}{E_{M-z}} = \frac{\varepsilon_M}{\varepsilon_I}$$

where E_{I-z} and E_{M-z} are the perpendicular components of the electric fields at the insulator and metal side of the interface, respectively, at the metal-dielectric interface, ε_M and ε_I are the complex permittivity of metal and insulator at ω_o , respectively. Furthermore, if the SERS substrate consists of periodic arrays of uniform plasmonic nanostructures, the ratio of average field penetration depths in dielectric and metal layers, t_I/t_M , can be further treated as a geometry-related parameter. Thus, we can further simplify eq. (S3) as:

$$\frac{I_{\text{MRS}}}{I_{\text{ERS}}} = \left| \frac{\varepsilon_M}{\varepsilon_I} \right|^4 \frac{\sigma_{\text{MRS}}(\omega_o, \Delta\omega_m)}{\sigma_{\text{ERS}}(\omega_o, \Delta\omega_e)} \frac{1}{|n_{e-h}(\Delta\omega_e) + 1|} \cdot r \cdot N = C \cdot r \cdot N \quad (\text{S4})$$

For typical SERS measurements under a CW excitation laser at room temperature, excluding r and N , all other terms in eq. (S4) can be grouped into a material-related parameter C , where $C =$

$$\left| \frac{\varepsilon_M}{\varepsilon_I} \right|^4 \frac{\sigma_{\text{MRS}}(\omega_o, \Delta\omega_m)}{\sigma_{\text{ERS}}(\omega_o, \Delta\omega_e)} \frac{1}{|n_{e-h}(\Delta\omega_e) + 1|}.$$

C does not depend on the incident laser intensity $I_o(\omega_o)$ as well as local field enhancement factors (g_I and g_M) at hotspots. Therefore, the ERS-calibrated MRS signals $I_{\text{MRS}}/I_{\text{ERS}}$ can be insensitive to variations of SERS EFs between different hotspots and thus can better quantify the concentration of analyte molecules (or more accurately, the density of molecular vibrational modes) compared to the directly measured I_{MRS} at hotspots on SERS substrates.

Fabrication of nanolaminate SERS substrates

Detailed fabrication steps are described elsewhere.¹⁵ First, a composite polydimethylsiloxane (PDMS) stamp having a diameter of 120 nm, a period of 400 nm, and a height of 150 nm, was prepared from a silicon wafer patterned with nanopillar structures by soft lithography.¹⁶ With the PDMS stamp, UV-curable polyurethane (PU) was used to fabricate a periodic nanopillar array by molding on a flexible and optically transparent polyester film. After UV curing for 10 min, an additional heat-curing process was performed in a convection oven at 80 °C overnight. Next, we deposited alternating layers of Au and SiO₂ by electron-beam evaporation. The four Au layers have the same thickness of 30 nm, and the thicknesses of three SiO₂ layers are nominally 6 nm, 8 nm, and 12 nm from bottom to top. Also, we deposited 1 nm of Cr between polymer nanopillar array and the first layer of Au, and 0.7 nm thick Ti between metal and insulator layers as adhesion layers.

Experimental setup

A confocal Raman microscope equipped with a 785 nm diode laser was used for SERS measurements. Before the measurement, the instrumental calibration was verified by the silicon peak at 520 cm⁻¹. All measurements were conducted in the backscattering geometric configuration at room temperature. Elastically scattered radiation at the wavelength corresponding to the laser line (Rayleigh scattering) is filtered out by a long-pass filter, while the rest of the collected light was guided through a multimode fiber (100 μm core diameter), acting as the pinhole for a confocal microscope, to a spectrometer. The backscattered photons were dispersed with a 300 groove/mm (750 nm blaze grating) and detected by a CCD camera, which was thermoelectrically cooled and maintained at -60 °C. For benzenethiol (BZT) experiments, ethanol-based 1 mmol/L BZT solution

was prepared, and samples were incubated overnight, followed by ethanol rinsing. For Rhodamine 6G (R6G) measurements, phosphate-buffered saline (PBS)-based R6G solutions with different concentrations were prepared. During the measurements, the samples were immersed in the solutions.

Effects of charge transfer at the molecule-metal interface on the ERS process

According to the orbital hybridization theory, two elementary quantum states with high spatial and energy overlaps can mix and form new hybridized states with an energy splitting. Therefore, spectrally-matched electronic states in sp-band at the metal surface can mix with HOMO-LUMO molecular states of the adsorbates to form new hybridized states. As the direct charge transfer happens, the e-h pair density deep below the Fermi level will decrease to reduce ERS signal intensity at the high-wavenumber region (relatively large $\Delta\omega_e$). On the other hand, when $\Delta\omega_e$ is small, the density of electron-hole pairs in metal nanostructures, $n_{e-h}(\Delta\omega_e) = \left| \exp\left(-\frac{\hbar\Delta\omega_e}{k_B T}\right) - 1 \right|^{-1}$, can become much higher than the molecule density at hotspots to dominate over the effects due to the charge transfer between the metal sp band and molecules. Experimental measurements agree that the ERS-pseudo peaks at the low wavenumber ($< 100 \text{ cm}^{-1}$) originating from the e-h pairs near the Fermi level show a similar intensity between plasmonic nanostructures with and without BZT molecules (Figure S2). Therefore, the ERS calibration's performance using the low-wavenumber ERS-pseudo peak is generally not affected by the adsorbed molecules and related charge transfer process.

Effects of molecule orientation variations on the SERS calibration performance

Since the ERS signals originate from the plasmonic metal nanostructures, the ERS signal intensity

is not susceptible to the analyte molecule orientation variations at plasmonic hotspots. In contrast, the MRS signals for specific vibrational modes of molecules at plasmonic hotspots depend on the molecule orientations. Notably, different vibration modes of the same molecule can have different Raman transition dipole moments $\hat{\mathbf{p}}$ in both directions and amplitudes, and the average Raman transition cross-section, $\overline{\sigma_{\text{MRS1}}}(\omega_o, \Delta\omega_{m1})$, for molecule ensembles depends on the average interaction Hamiltonian $\overline{\hat{\mathbf{H}}} = -\overline{\hat{\mathbf{p}} \cdot \hat{\mathbf{E}}}$, where $\hat{\mathbf{E}}$ is the local electric field at plasmonic hotspots. Therefore, the MRS calibrated ratiometric value, $\frac{I_{\text{MRS1}}}{I_{\text{MRS2}}}$, between two vibrational modes depends on the average response from molecule ensembles at many plasmonic hotspots within the excitation laser beam spot in SERS measurements. To examine the effects of molecule orientation variations on the MRS calibrated values, we can look at the following relations, expressed as

$$\frac{I_{\text{MRS1}}}{I_{\text{MRS2}}} \propto \frac{\overline{\sigma_{\text{MRS1}}}(\omega_o, \Delta\omega_{m1})}{\overline{\sigma_{\text{MRS2}}}(\omega_o, \Delta\omega_{m2})} \propto \frac{\overline{\hat{\mathbf{p}}_{m1} \cdot \hat{\mathbf{E}}}}{\overline{\hat{\mathbf{p}}_{m2} \cdot \hat{\mathbf{E}}}} \propto \frac{\overline{r_{m1}}}{\overline{r_{m2}}},$$

where $\overline{r_{m1}}$ and $\overline{r_{m2}}$ are the molecule ensemble-averaged orientation factors ($0 \leq r \leq 1$) for MRS1 and MRS2 modes, respectively. Based on the above analysis, we can expect that the MRS calibration process cannot remove either spatial or temporal variations in molecule orientations for molecule ensembles at plasmonic hotspots. Furthermore, compared to the ERS calibration process, the MRS calibration process is generally more susceptible to molecule orientation variations and performs less well, also as shown in the

following relations: $\frac{\delta I_{\text{MRS1}}}{\delta I_{\text{MRS2}}} \propto \frac{\delta \overline{r_{m1}}}{\delta \overline{r_{m2}}} > \frac{\delta I_{\text{MRS1}}}{I_{\text{ERS}}} \propto \delta \overline{r_{m1}} \cdot (0 \leq \delta \overline{r_{m1}} \leq 1, 0 \leq \delta \overline{r_{m2}} \leq 1)$

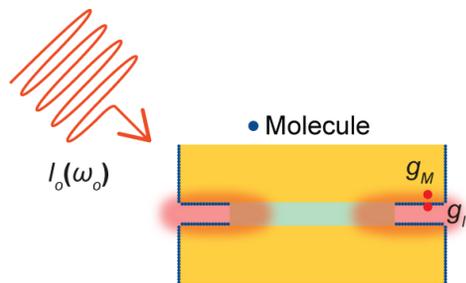


Figure S1. Schematic illustration of a single unit cell of the nanolaminate SERS substrates.

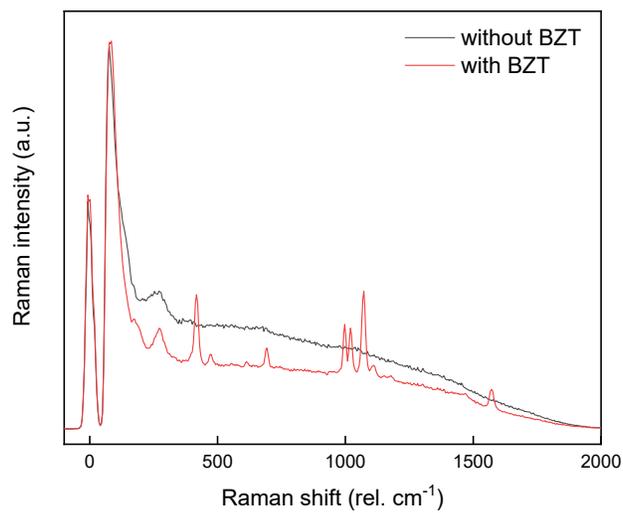


Figure S2. Measured SERS spectra of plasmonic nanostructures with and without BZT molecules (Reproduced from Figure 1F).

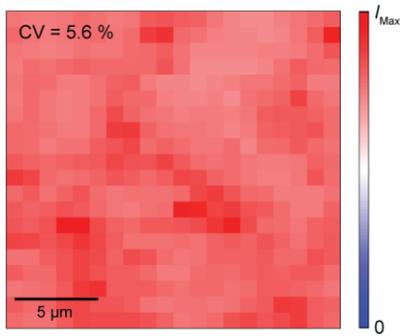


Figure S3. 2D Raman mapping of BZT on the nanolaminate SERS substrate without sample buckling.

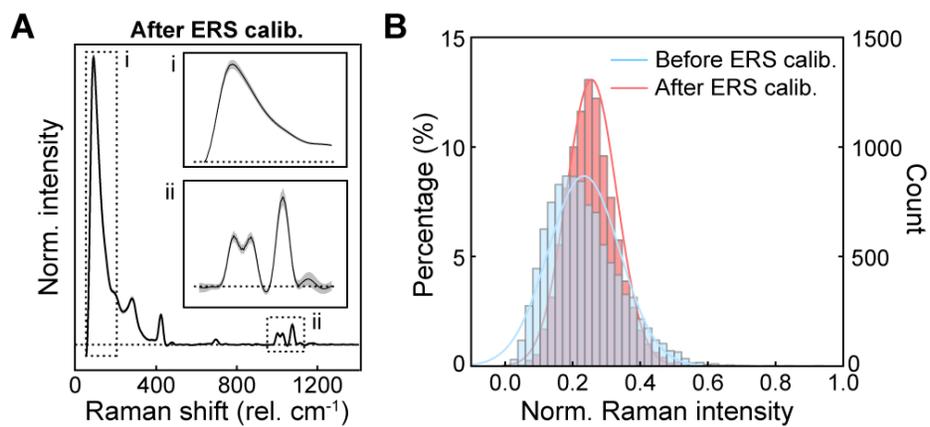


Figure S4. (A) Average BZT SERS spectrum with one SD (gray regions) from 10,000 pixels after ERS calibration. (B) Histograms of BZT SERS signal intensities before and after ERS calibration.

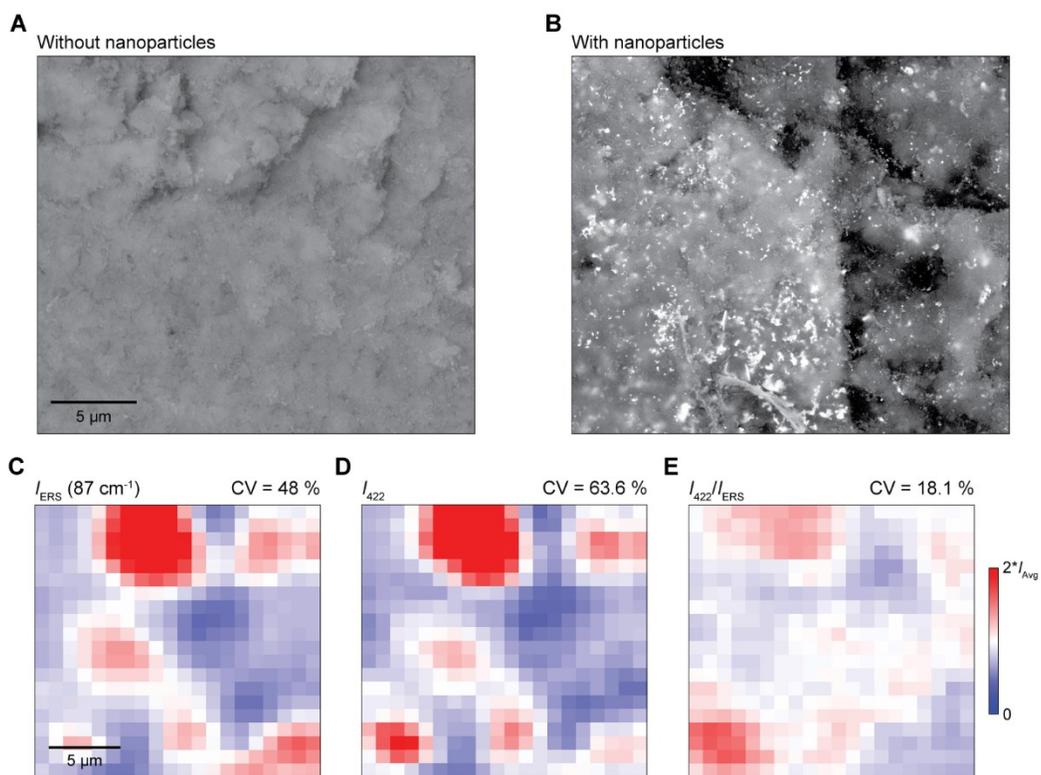


Figure S5. SEM images of bacteria cellulose (A) without and (B) with gold nanoparticles. 2D Raman images of BZT for (C) I_{ERS} , (D) I_{422} , and (E) I_{422}/I_{ERS} .

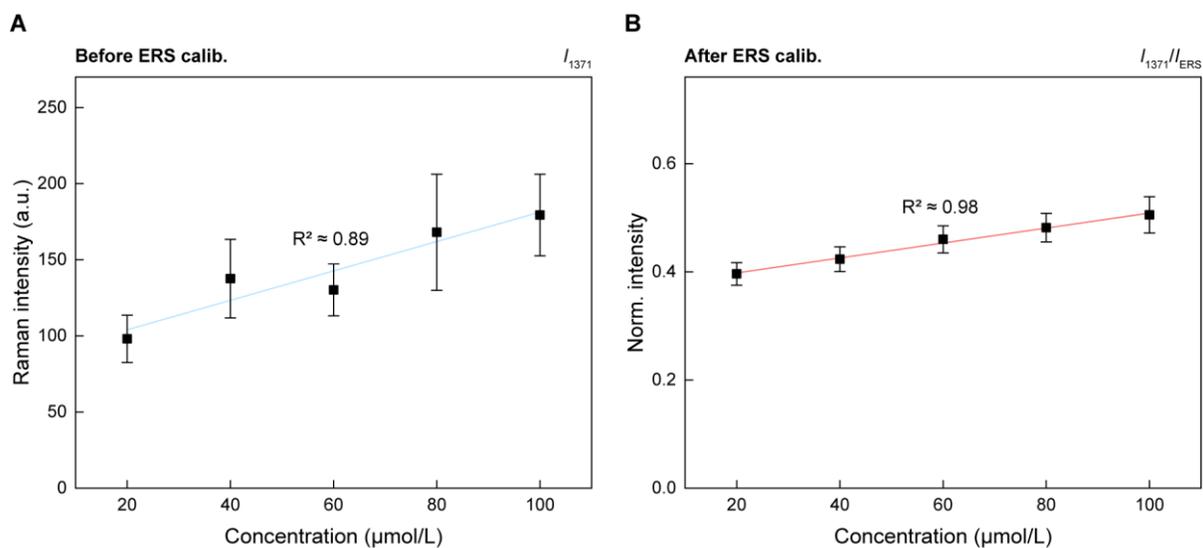


Figure S6. Working curves of solution-based R6G molecules with different concentrations from 20 $\mu\text{mol/L}$ to 100 $\mu\text{mol/L}$ using 1371 cm^{-1} (A) before and (B) after ERS calibration. The error bars show one standard deviation from 400 pixels.

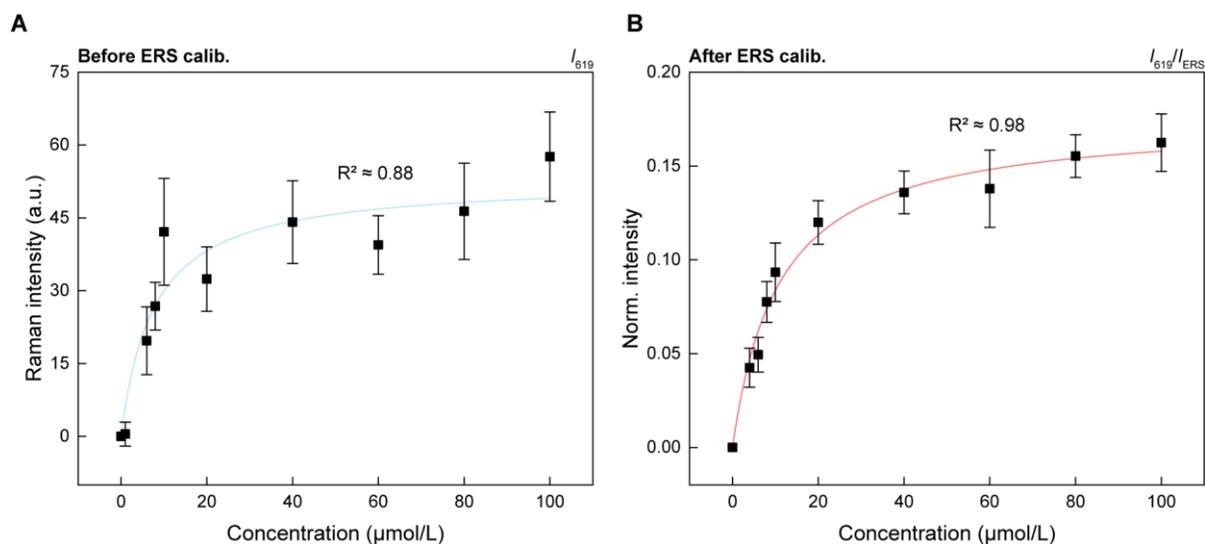


Figure S7. Working curves of solution-based R6G molecules with different concentrations from 4 $\mu\text{mol/L}$ to 100 $\mu\text{mol/L}$ using 619 cm^{-1} (A) before and (B) after ERS calibration. The error bars show one standard deviation from 400 pixels.

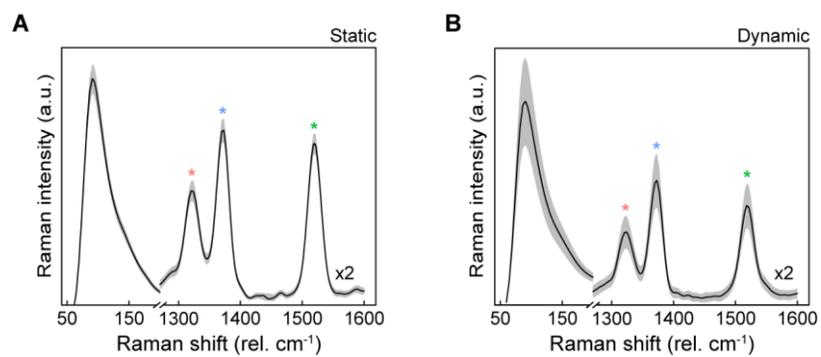


Figure S8. Temporally averaged Raman spectra with one SD (gray regions) from single-spot time-resolved SERS measurements over 300 s under (A) static and (B) dynamic laser excitation. Intensities in the MRS region between 1300 cm^{-1} and 1600 cm^{-1} are multiplied by two for clarity.

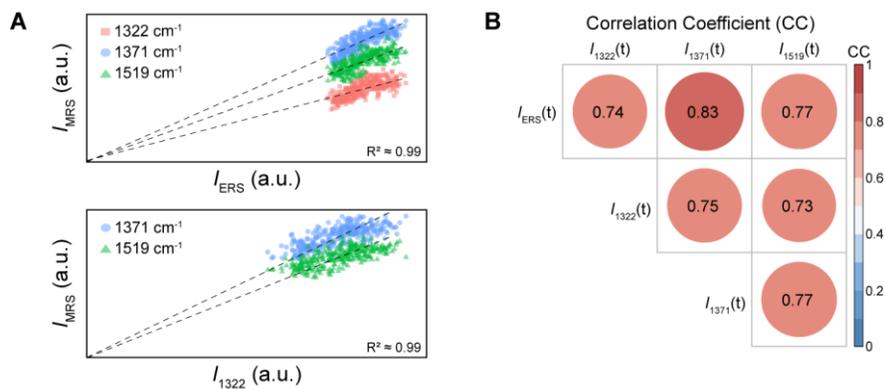


Figure S9. (A) The scatter plots of I_{MRS} as a function of I_{ERS} (top) and I_{1322} (bottom) under static laser excitation. (B) A matrix of calculated correlation coefficients among ERS and MRS signals under static laser excitation.

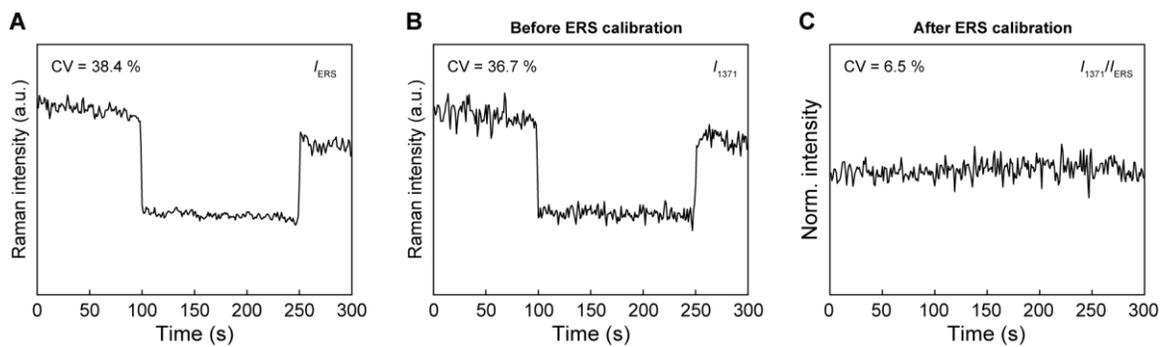


Figure S10. Time-trajectories of (A) ERS and MRS signals using 1371 cm^{-1} (B) before and (C) after ERS calibration with abrupt laser power changes between 0.25 mW and 0.5 mW.

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