Supplementary information for

A robust SERS calibration using pseudo-internal intensity reference

Meng Zhang, Jingran Yang, Longkun Yang and Zhipeng Li**

Beijing Key Laboratory of Nano-Photonics and Nano-Structure (NPNS), Department of Physics, Capital Normal University, Beijing 100048, China.

**. Co-corresponding author. E-mail: zpli@cnu.edu.cn E-mail: lkyang@cnu.edu.cn*

Calculate the standard deviation of normalized SERS intensity

The standard deviation propagation equation can be expressed as:

$$S_N = \sqrt{\left(\frac{\partial f}{\partial I_1}\right)^2 {S_{I_1}}^2 + \left(\frac{\partial f}{\partial I_2}\right)^2 {S_{I_2}}^2} \tag{1}$$

where I_1,I_2denote the peak intensity and the reference intensity, and S_{I_1},S_{I_2}represent the standard deviation corresponding to the I_1,I_2, respectively. In this work, the expression of function f is as follows：

$$f(I_1,I_2) = \frac{I_1}{I_2} \tag{2}$$

Take equation (2) into equation (1) can get the error propagation equation:

$$S_N = \sqrt{\left(\frac{S_{I_1}}{I_2}\right)^2 + \left(\frac{I_1 S_{I_2}}{{I_2}^2}\right)^2} \tag{3}$$

We can get the error after propagation by bring the peak intensities and old error into the equation (3).

Calculate the standard deviation of the predicted concentration

The relationship between SERS intensity and concentration can be expressed as

$$I = a + b\lg C \tag{4}$$

The concentration expression from equation (4) is given by:

$$\bar{C} = 10^{\frac{\bar{I} - a}{b}} \tag{5}$$

Taking equation (5) into equation (1) can get the error propagation equation as:

$$SD_c = 10^{\frac{\bar{I} - a}{b}} \times \frac{ln10}{b} SD_I \tag{6}$$

That is

$$SD_c = \overline{C} \times \frac{ln10}{b} SD_I \tag{7}$$

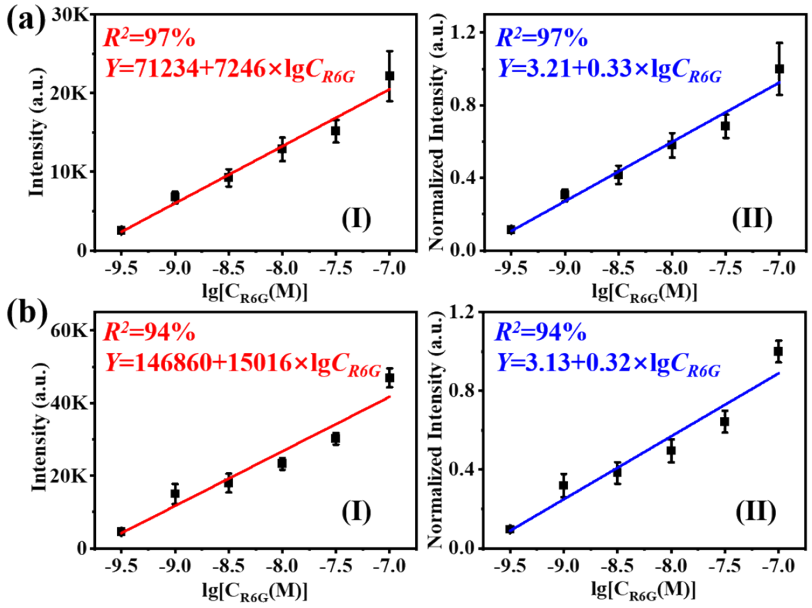

Fig. S1 (a) and (b) SERS intensity at 771 cm^{-1} as a function of R6G concentration (1.0×10^{-7} M-3.0×10^{-10} M) obtained in two independent batches of experiments. Panel (I) shows the SERS intensity before normalization, where the error bars denote the standard deviation of SERS intensity. Panel (II) shows the SERS intensity after normalization, where the error bars are obtained by the error propagation.

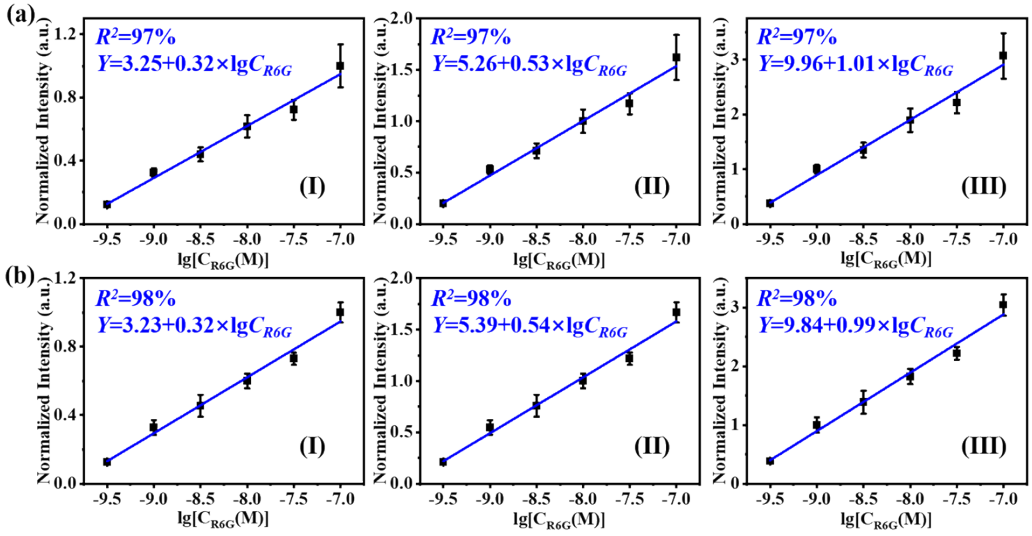

Fig. S2 (a) Normalize the SERS intensity of Fig. 2a of Panel I using the intensity reference of concentration (I) 1.0×10^{-7} M, (II) 1.0×10^{-8} M and (III) 1.0×10^{-9} M; (b) Normalize the SERS intensity of Fig. 2b of Panel I using the intensity reference of concentration (I) 1.0×10^{-7} M, (II) 1.0×10^{-8} M and (III) 1.0×10^{-9} M.

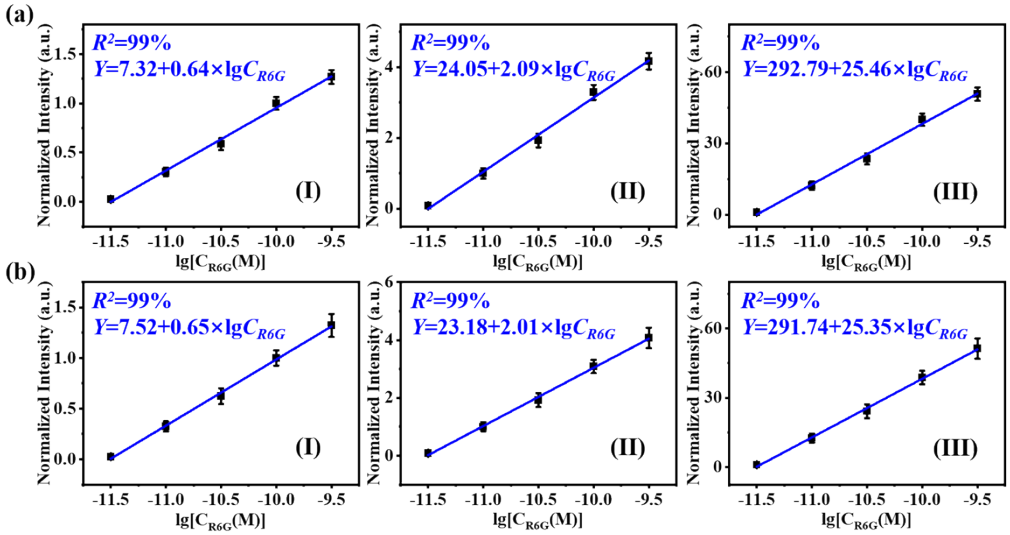

Fig.S3 (a) Normalize the SERS intensity of Fig. 3a of Panel I using the intensity reference of concentration (I) 1.0×10^{-10} M, (II) 1.0×10^{-11} M and (III) 3.0×10^{-12} M; (b) Normalize the SERS intensity of Fig. 3b of Panel I using the intensity reference of concentration (I) 1.0×10^{-10} M, (II) 1.0×10^{-11} M and (III) 3.0×10^{-12} M.

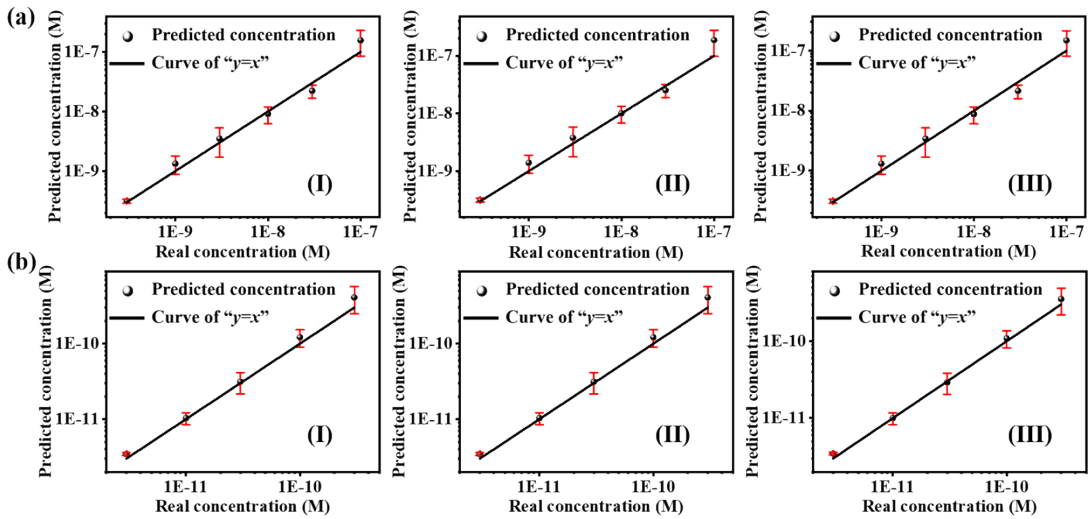

Fig.S4 (a) The predicted concentration of the R6G molecule by using the intensity of (I) 1.0×10^{-7} M, (II) 1.0×10^{-8} M, (II)1.0×10^{-9} M as the pseudo-internal standard; (b) The predicted concentration of the R6G molecule by using the intensity of (I) 1.0×10^{-10} M, (II) 1.0×10^{-11} M, (II) 3.0×10^{-12} M as the pseudo-internal standard.

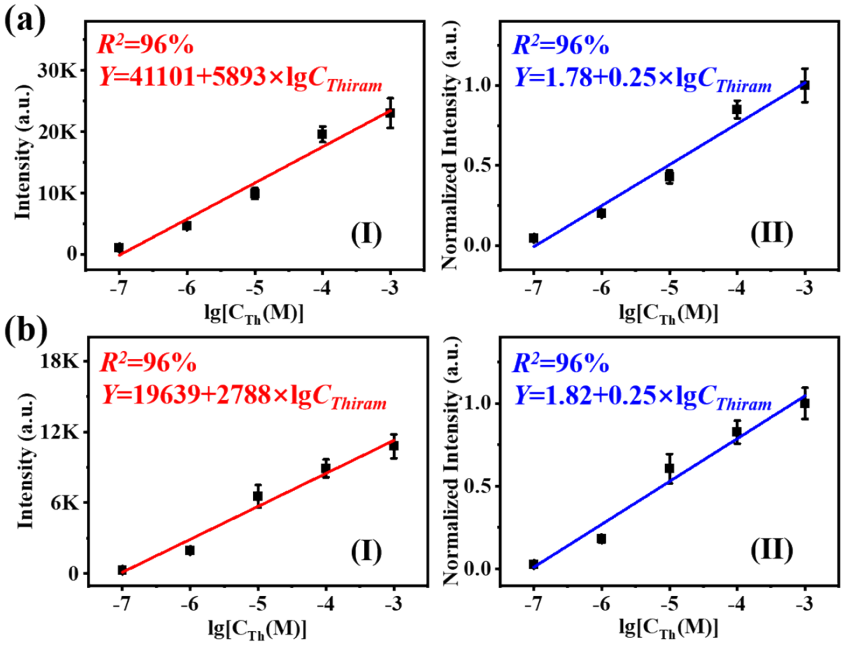

Fig.S5 (a) and (b) SERS intensity at 1387 cm^{-1} as a function of Thiram concentration (1.0×10^{-7} M-1.0×10^{-3} M) obtained in two independent batches of experiments. Panel I show the SERS intensity before normalization, where the error bars denote the standard deviation of SERS intensity. Panel II shows the SERS intensity after normalization, where the error bars are obtained by the error propagation.

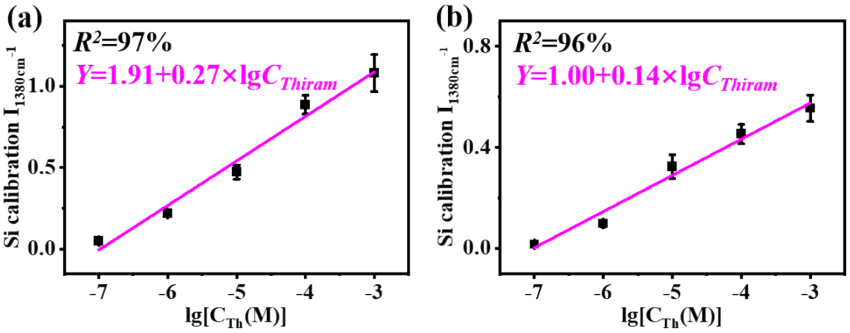

Fig.S6 (a) The Thiram SERS intensities of Panel I of Fig. S5a calibrated with silicon peaks. (b) The Thiram SERS intensities of Panel I of Fig. S5b calibrated with silicon peaks.

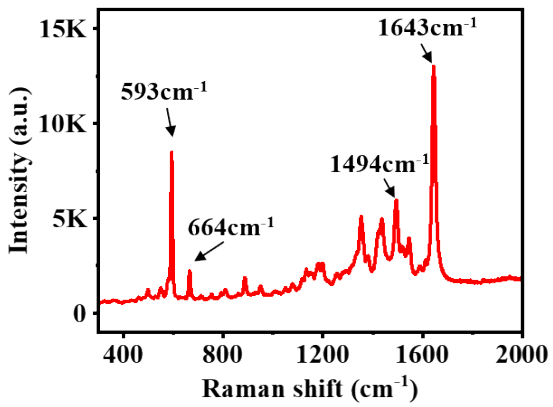

Fig. S7 The SERS spectra of NB molecule.

In Fig.S8, the RSDs of R6G SERS intensity (1.0×10^{-7} M) from the two batches were 10.2% and 5.3%, respectively.

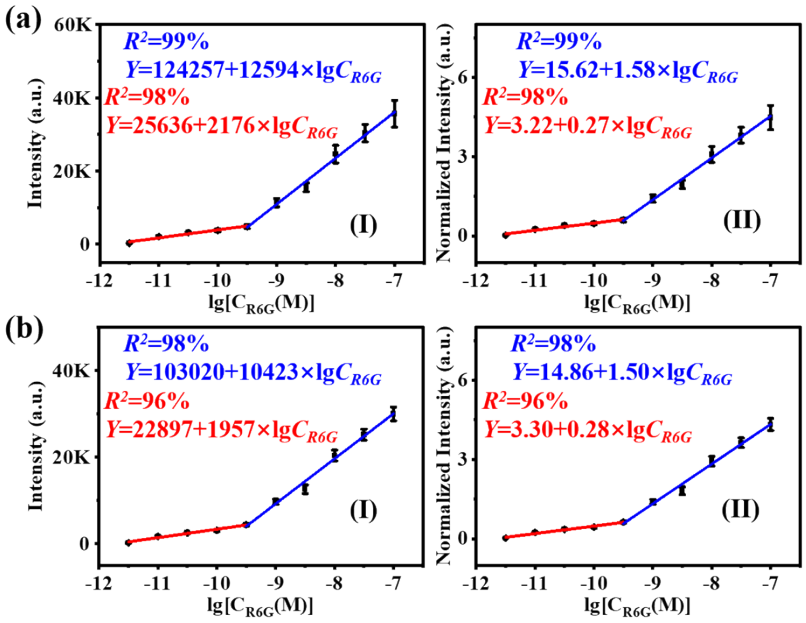

Fig. S8 (a) and (b) SERS intensity at 610 cm^{-1} as a function of R6G concentration (1.0×10^{-7} M-3.0×10^{-12} M) obtained in two independent batches of experiments. Panel I show the SERS intensity before normalization, where the error bars denote the standard deviation of SERS intensity. Panel II shows the SERS intensity after normalization with NB, where the error bars are obtained by the error propagation.

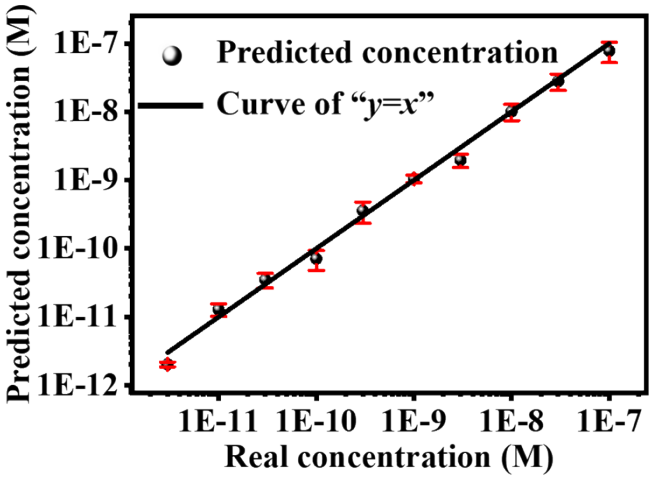

Fig. S9 Concentration analysis of R6G molecules using the normalized calibration curve.

All the SERS spectra in this paper

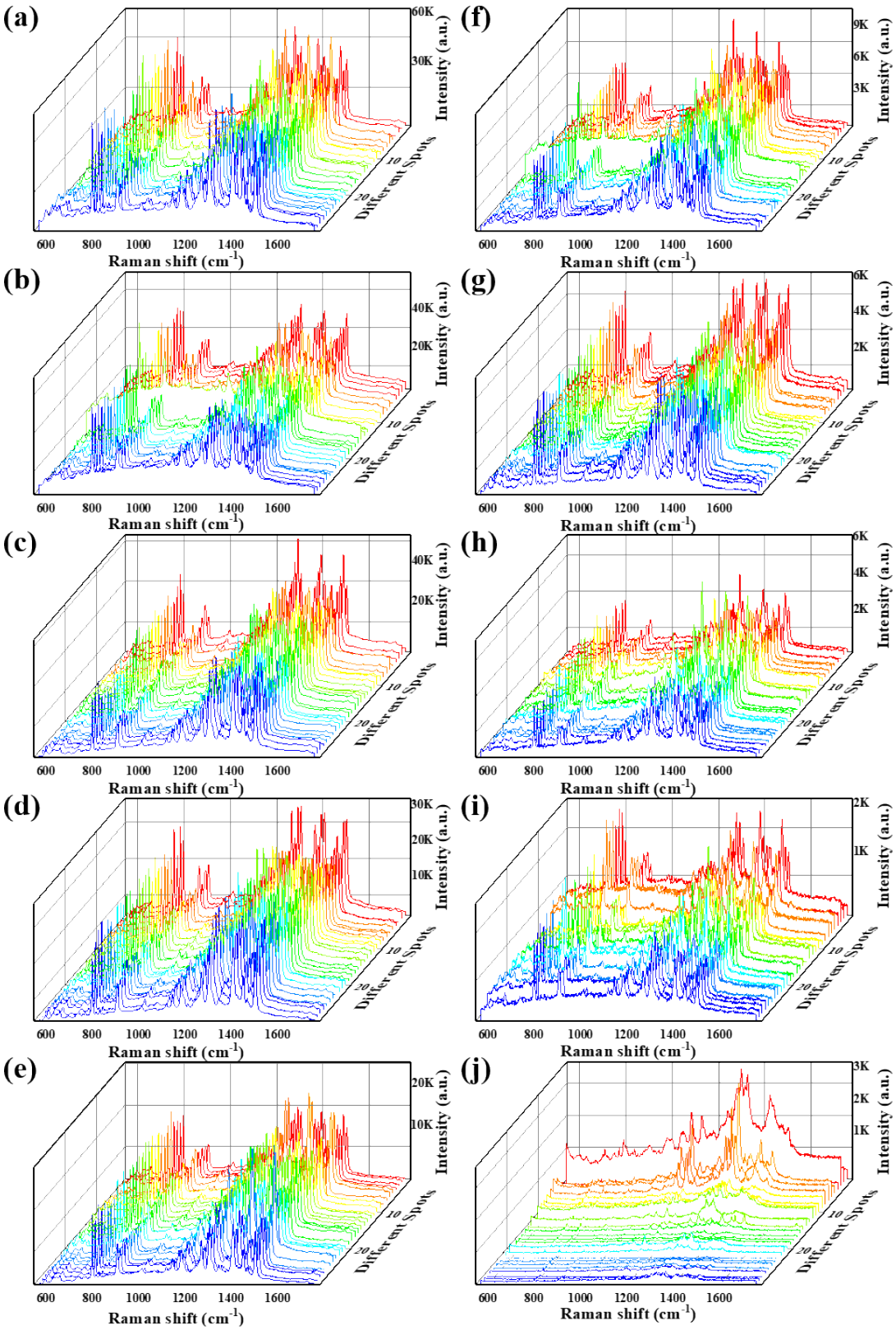

Fig.S10 The SERS spectra with R6G concentration (a) 1.0×10^{-7} M, (b) 3.0×10^{-8} M, (c) 1.0×10^{-8} M, (d) 3.0×10^{-9} M, (e) 1.0×10^{-9} M, (f) 3.0×10^{-10} M, (g) 1.0×10^{-10} M, (h) 3.0×10^{-11} M, (i) 1.0×10^{-11} M, (j) 3.0×10^{-12} M. (a)-(e) corresponding to the Pannal I of Fig. 2a and (f)-(j) corresponding to the Pannal I of Fig. 3a.

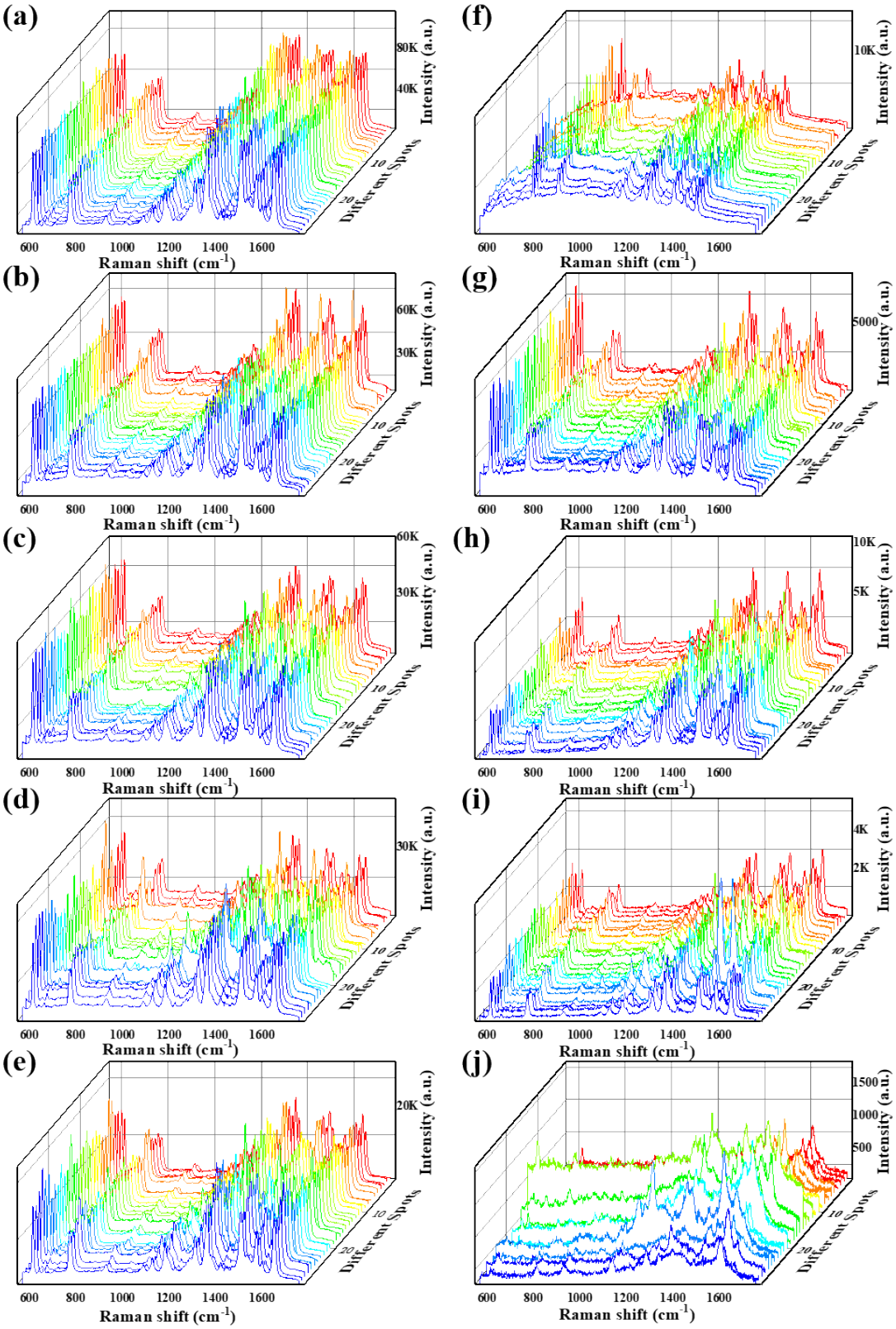

Fig.S11 The SERS spectra with R6G concentration (a) 1.0×10^{-7} M, (b) 3.0×10^{-8} M, (c) 1.0×10^{-8} M, (d) 3.0×10^{-9} M, (e) 1.0×10^{-9} M, (f) 3.0×10^{-10} M, (g) 1.0×10^{-10} M, (h) 3.0×10^{-11} M, (i) 1.0×10^{-11} M, (j) 3.0×10^{-12} M. (a)-(e) corresponding to the Pannal I of Fig. 2b and (f)-(j) corresponding to the Pannal I of Fig. 3b.

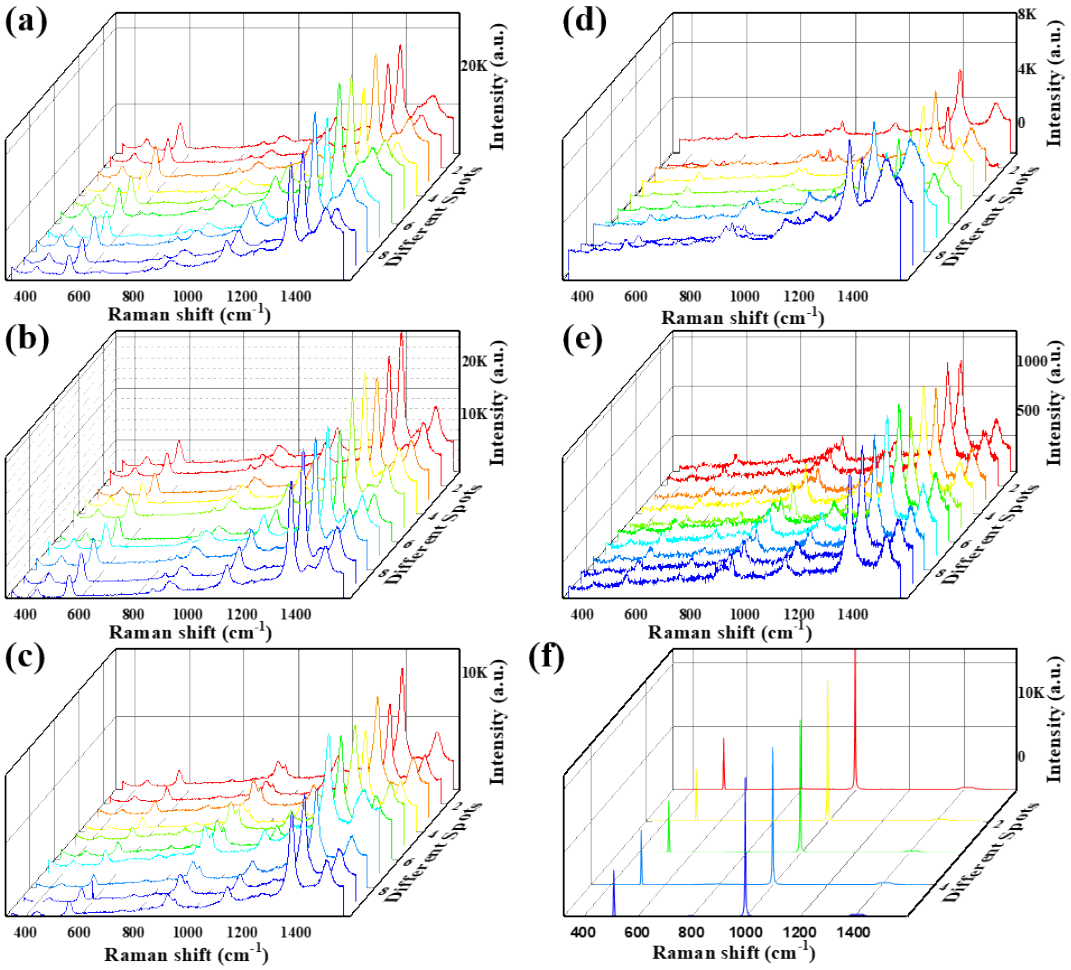

Fig.S12 The SERS spectra with thiram concentration (a) 1.0×10^{-3} M, (b) 1.0×10^{-4} M, (c) 1.0×10^{-5} M, (d) 1.0×10^{-6} M, (e) 1.0×10^{-7} M, which corresponding to the Pannal I of Fig. S5a. (f) Silicon peaks measured with external standard calibration.

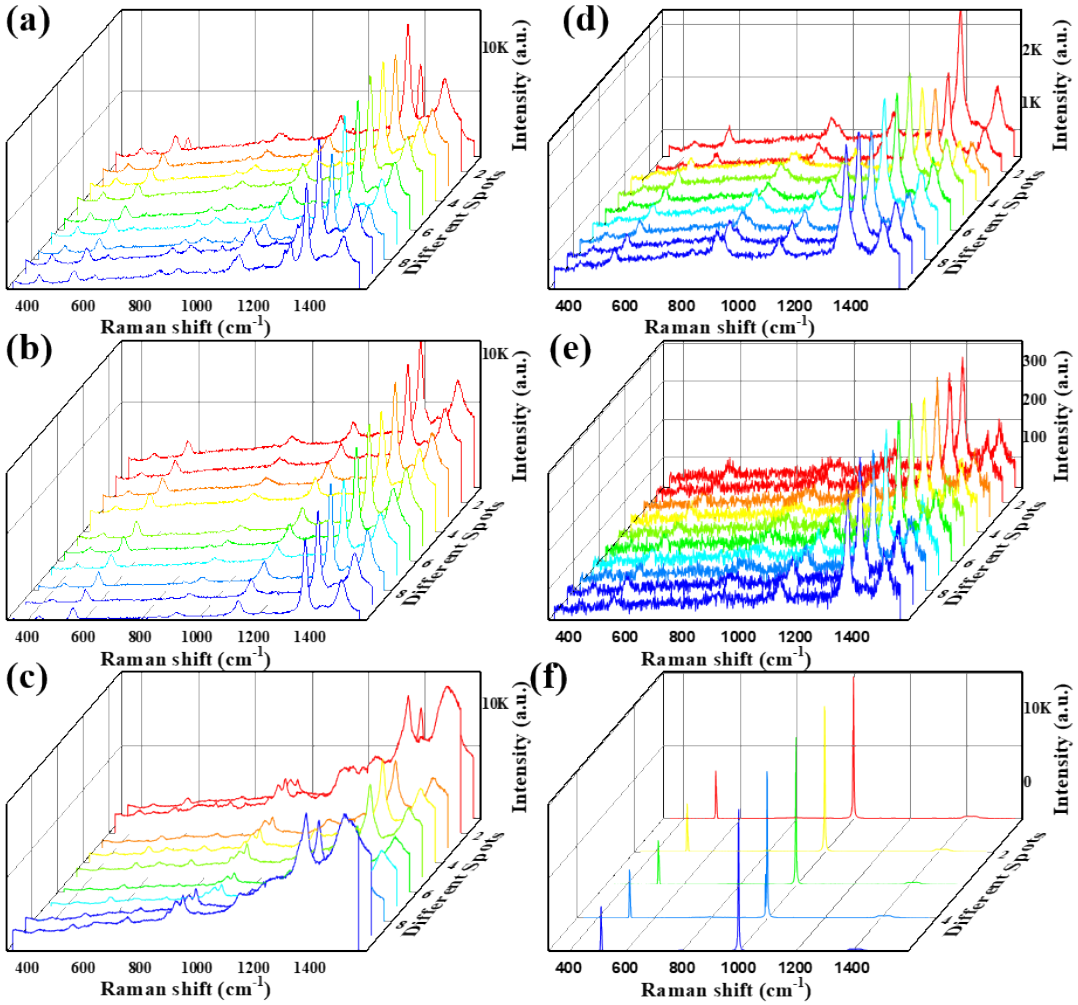

Fig.S13 The SERS spectra with thiram concentration (a) 1.0×10^{-3} M, (b) 1.0×10^{-4} M, (c) 1.0×10^{-5} M, (d) 1.0×10^{-6} M, (e) 1.0×10^{-7} M, which corresponding to the Pannal I of Fig. S5b. (f) Silicon peaks measured with external standard calibration.

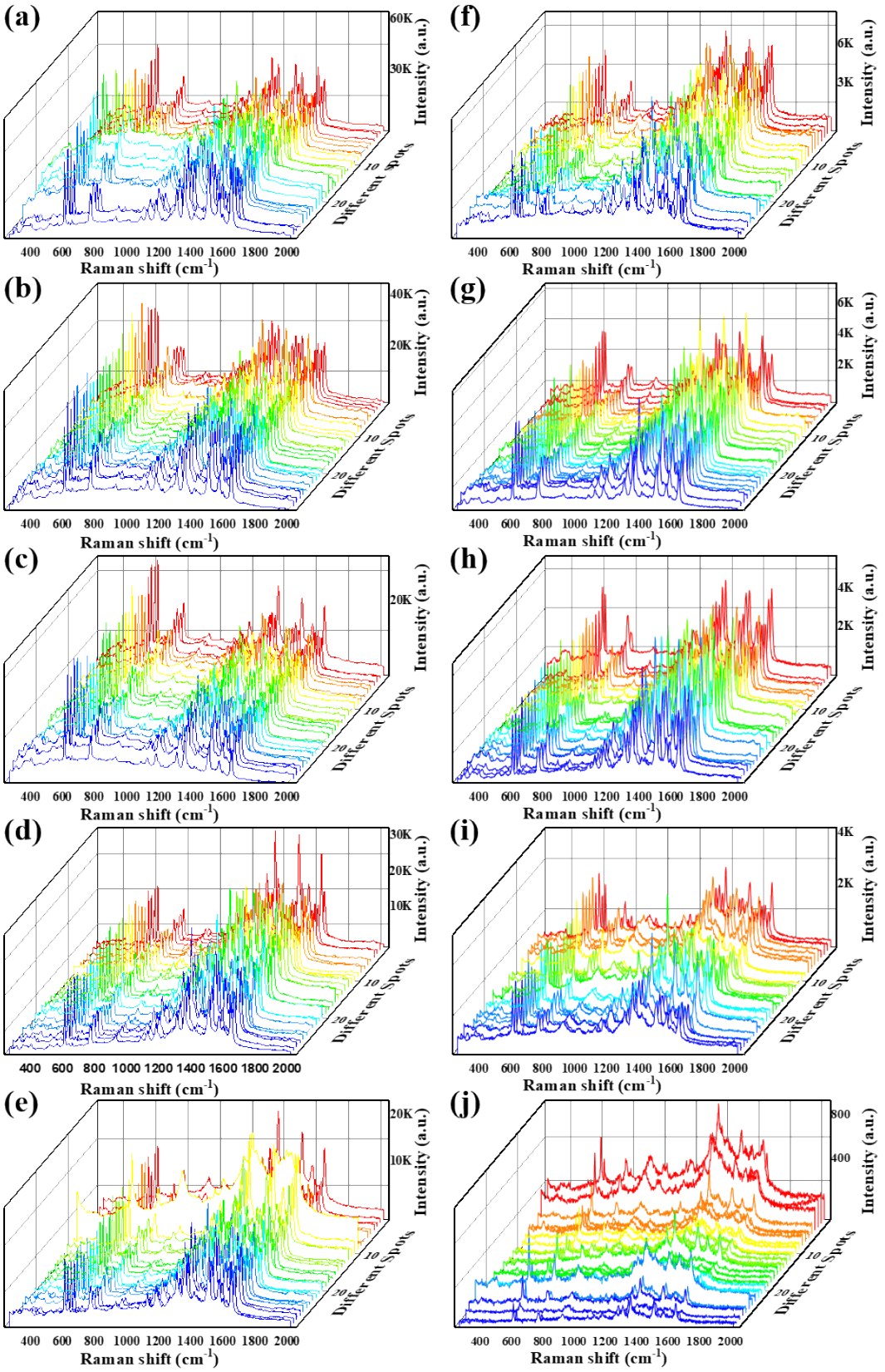

Fig.S14 The SERS spectra with R6G concentration (a) 1.0×10^{-7} M, (b) 3.0×10^{-8} M, (c) 1.0×10^{-8} M, (d) 3.0×10^{-9} M, (e) 1.0×10^{-9} M, (f) 3.0×10^{-10} M, (g) 1.0×10^{-10} M, (h) 3.0×10^{-11} M, (i) 1.0×10^{-11} M, (j) 3.0×10^{-12} M, which corresponding to the Pannal I of Fig. S8a.

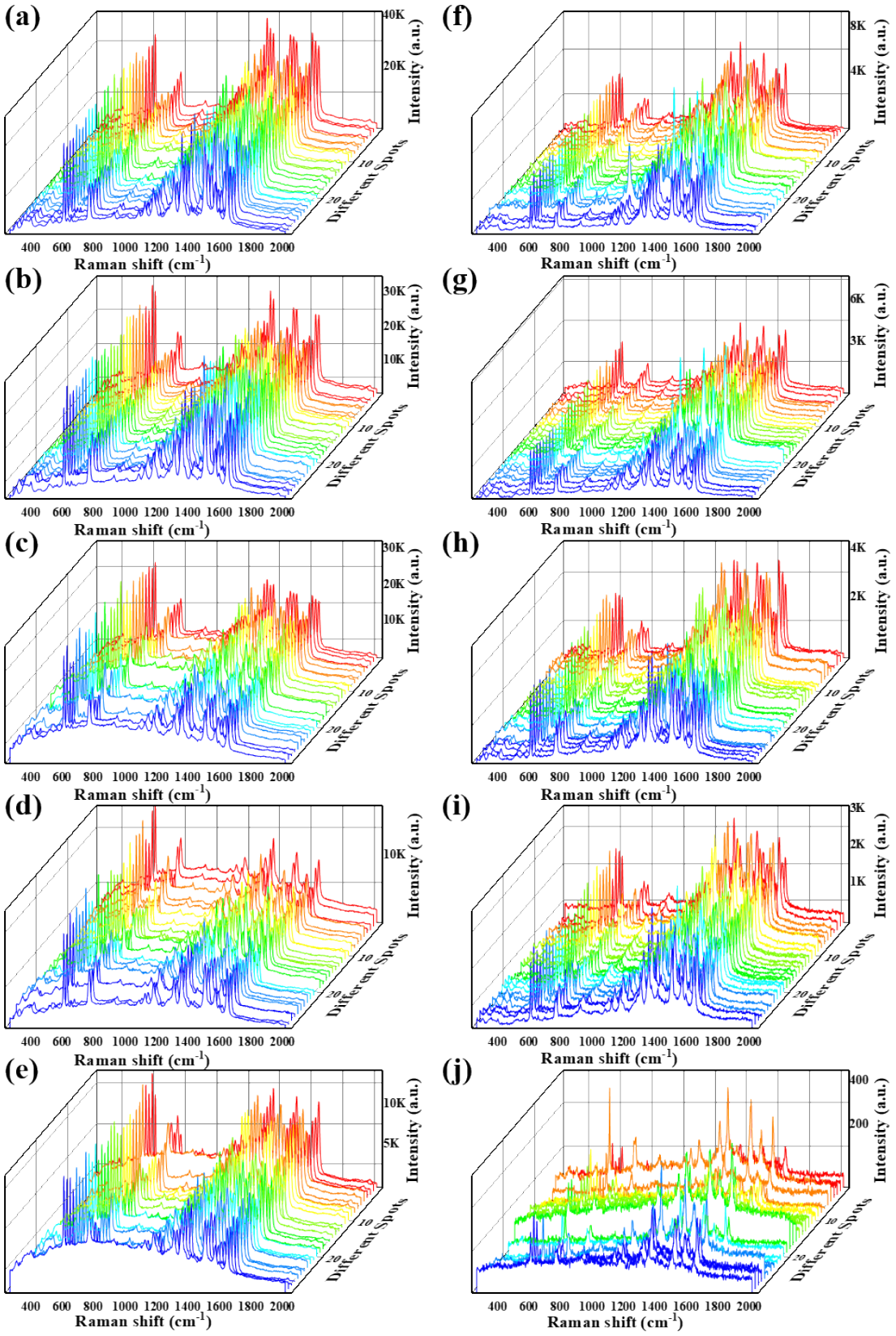

Fig.S15 The SERS spectra with R6G concentration (a) 1.0×10^{-7} M, (b) 3.0×10^{-8} M, (c) 1.0×10^{-8} M, (d) 3.0×10^{-9} M, (e) 1.0×10^{-9} M, (f) 3.0×10^{-10} M, (g) 1.0×10^{-10} M, (h) 3.0×10^{-11} M, (i) 1.0×10^{-11} M, (j) 3.0×10^{-12} M, which corresponding to the Pannal I of Fig. S8b.

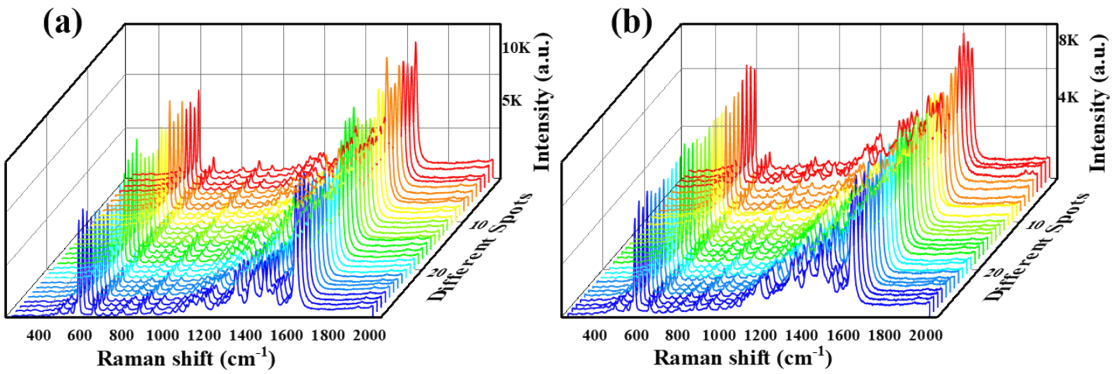

Fig.S16 The SERS spectra with NB concentration 1.0×10^{-8} M. (a) used to calibrate the spectra in Fig. S14, (b) used to calibrate the spectra in Fig. S15.