Supporting information for

Mapping gold nanoparticles on and in edible leaves in situ using surface enhanced Raman spectroscopy

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Table S1. Characterization information of Au NPs

Sample No.	Diameter (TEM)/nm	Hydrodynamic diameter/nm	Zeta Potential/mV	UV-vis absorbance (λ_{max}/nm)
1	15.4±0.9	21.2±0.5	-31.9±1.4	520
2	35.3±2.6	46.9±0.4	-33.3±1.7	528
3	78.8±6.4	88.6±0.9	-21.1±0.7	561
4	122.7±8.2	129.3±1.3	-22.3±1.3	604

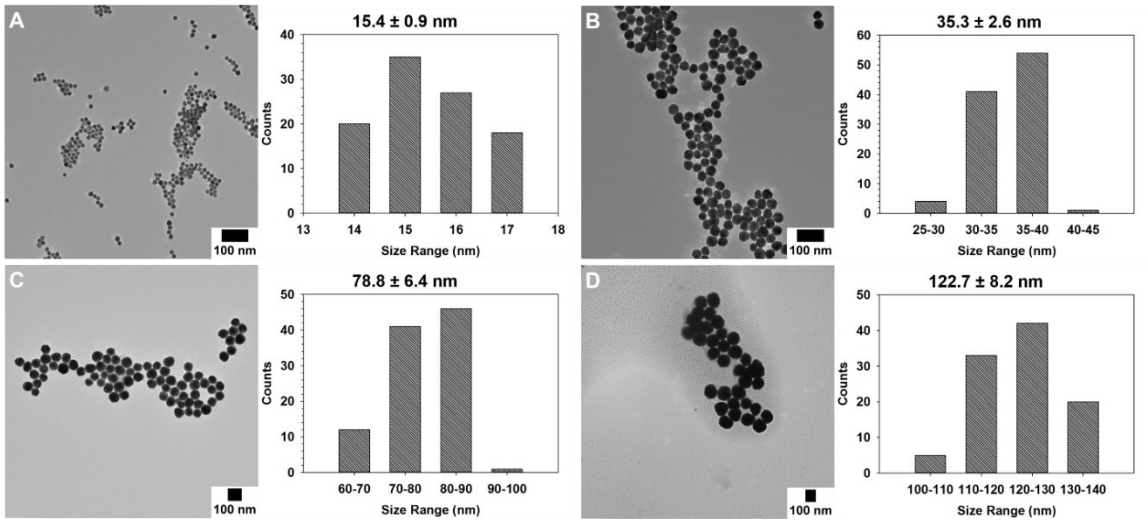

Figure S1. TEM images of four different sizes of Au NPs synthesized in this study and their size distributions. (a), 15.4 ± 0.9 nm; (b), 35.3 ± 2.6 nm; (c), 78.8 ± 6.4 nm; (d), 122.7 ± 8.2 nm.

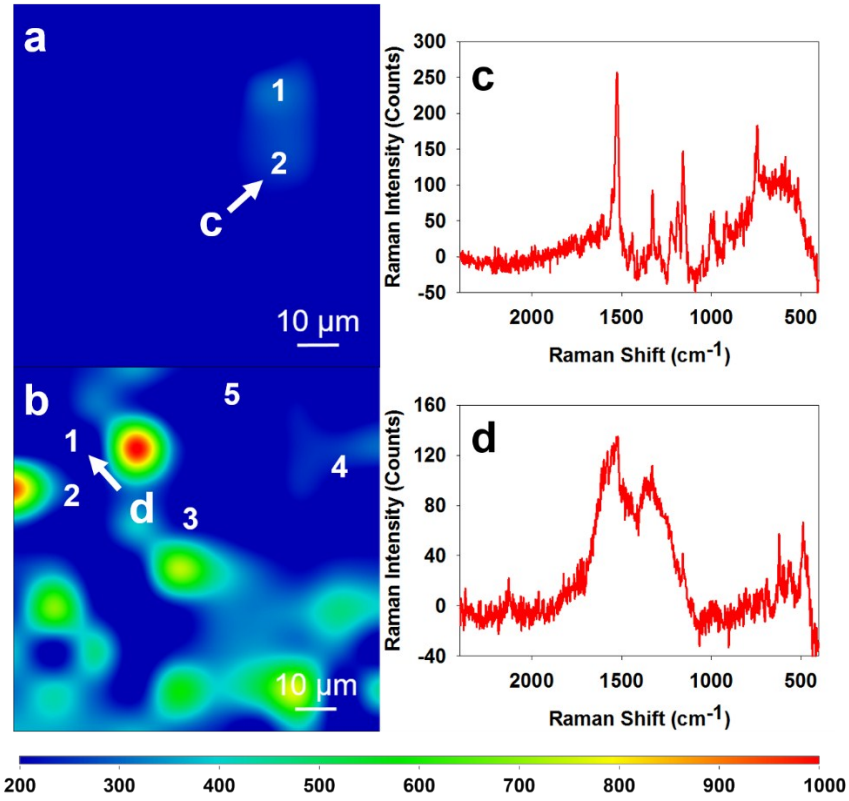

Figure S2. Estimation of false positive (2%) and false negative (5%) of the mapping method with the baseline set at 200 counts based on the 1525 cm^{-1} peak. (a) 2D Raman mapping of spinach leaf without Au NPs. Two out of 100 points had higher than 200 counts, (b) 2D Raman mapping of the spinach leaf with Au NPs. Five out of 100 points contain characteristic peaks of Au NPs but had lower than 200 counts, (c) representative spectrum of the false positive signal, (d), representative spectrum of the false negative signal.

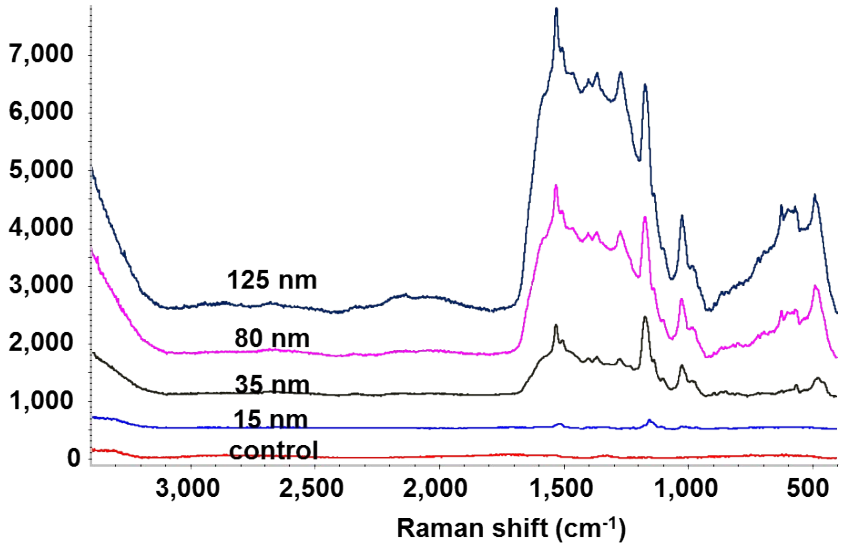

Figure S3. SERS spectra of pigments (chlorophylls and carotenoids) extracted from spinach leaves mixed with Au NPs of different sizes (15-125 nm). The detailed extraction was adapted by a published protocol[1] as follows. 100 μL extracted solutions (500 mg L^{-1}) were mixed with 100 μL Au NPs at 50 mg L^{-1} for 30 min, then the Au NPs were deposited on a gold coated glass slide for Raman measurement. Compared with control (just pigments without Au NPs), all the Au NPs show enhanced signals. The SERS spectra obtained was found similar to the *in situ* SERS spectra of Au NPs on and in spinach leaves, indicating the Au NPs were interacted with plant pigments on and in spinach leaves. 15 nm Au NPs shows the least enhancement, while the 125 nm Au NPs show the most enhancements. The similar size dependent SERS enhancement was also reported in other study.[2]

One gram of fresh spinach leaves was cut into small pieces in a mortar along with 0.5 g of sodium sulfate and 2.0 mL of acetone. Next, this mixture was transferred to a centrifuge tube with 2.0 mL of hexane and was thoroughly shake. After that, 2.0 mL of water was added into the centrifuge tube and the top organic layer was transferred into a clean test tube. Finally, another 1.0 mL of hexane was added to the previous centrifuge tube that contains the aqueous layer. The top organic layer was separate again and was transferred to the test tube.

1. Pavia, D.L., Lampman, G.M., Kriz, G.S., and Engel, R. . Isolation of Chlorophyll and Carotenoid Pigments from Spinach. *Introd. to Org. Lab. Tech. A Microscale Approach 3rd Ed.* 1–7 (1999).

2. Njoki, P. N. *et al.* Size Correlation of Optical and Spectroscopic Properties for Gold Nanoparticles. *J. Phys. Chem. C* **111,** 14664–14669 (2007).

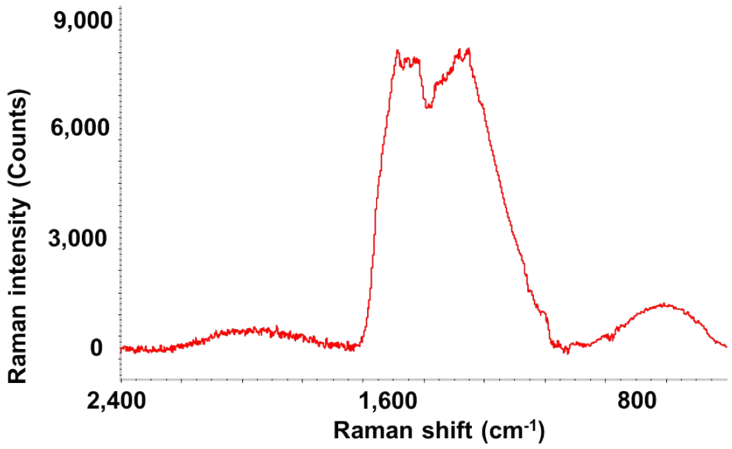

Figure S4. SERS spectrum of Au NPs aggregates dried on a gold coated slide. A broad peak over 1000-1700 cm^{-1} was observed, which is similar to the *in situ* spectra observed from Au NPs on and in spinach leaves. The intensity of the signals on a gold slide was higher than that on and in spinach leaves because of the better reflectivity of the gold slide.

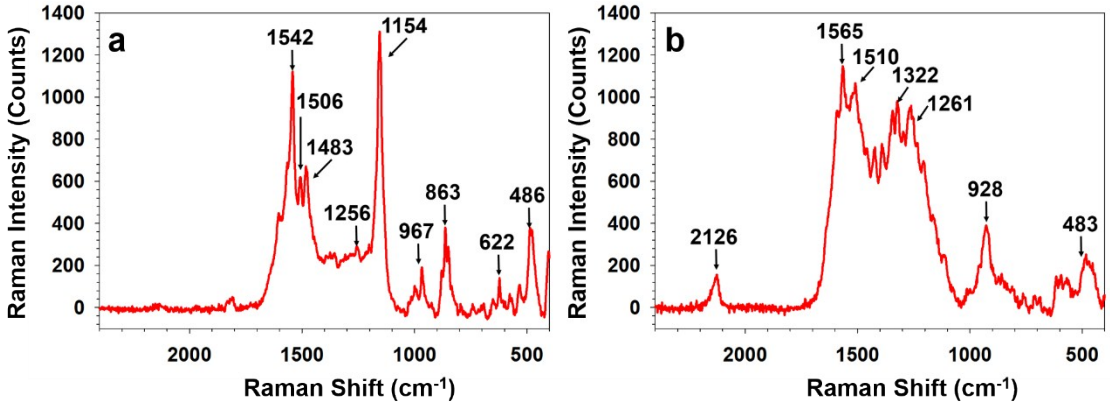

Figure S5. SERS spectra of different spots in spinach leaves at 20 μm depth *in situ*. They show different patterns combining the characteristic peaks of carotenoids and chlorophylls, as well as the sulfur peaks, which demonstrate the strong interactions with plant pigments and sulfur containing compounds.

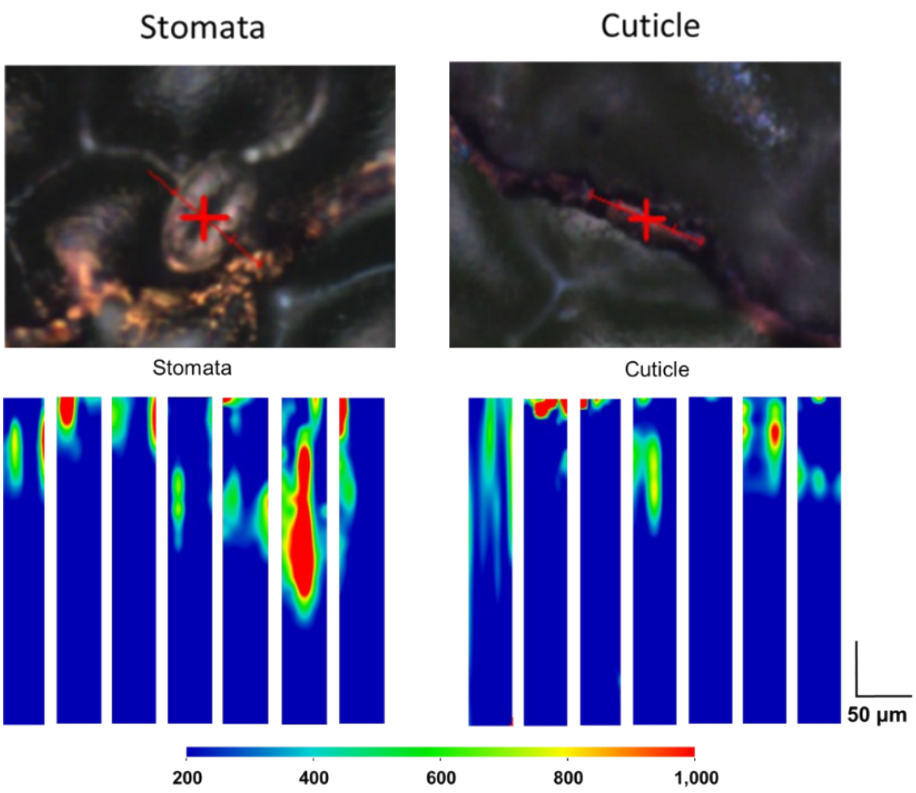

Figure S6. Optical images of representative selected areas for studying stomata and cuticle penetration, and SERS mapping of penetration depth profiles of 35 nm Au NPs through randomly picked stomata and cuticle on spinach leaf surfaces. Both of these two penetration pathways show variations in term of penetration depth, and there is no statistical difference between them. Stomata may allow more Au NPs to penetrate in some cases, as indicated by intense signals observed in the depth profile.

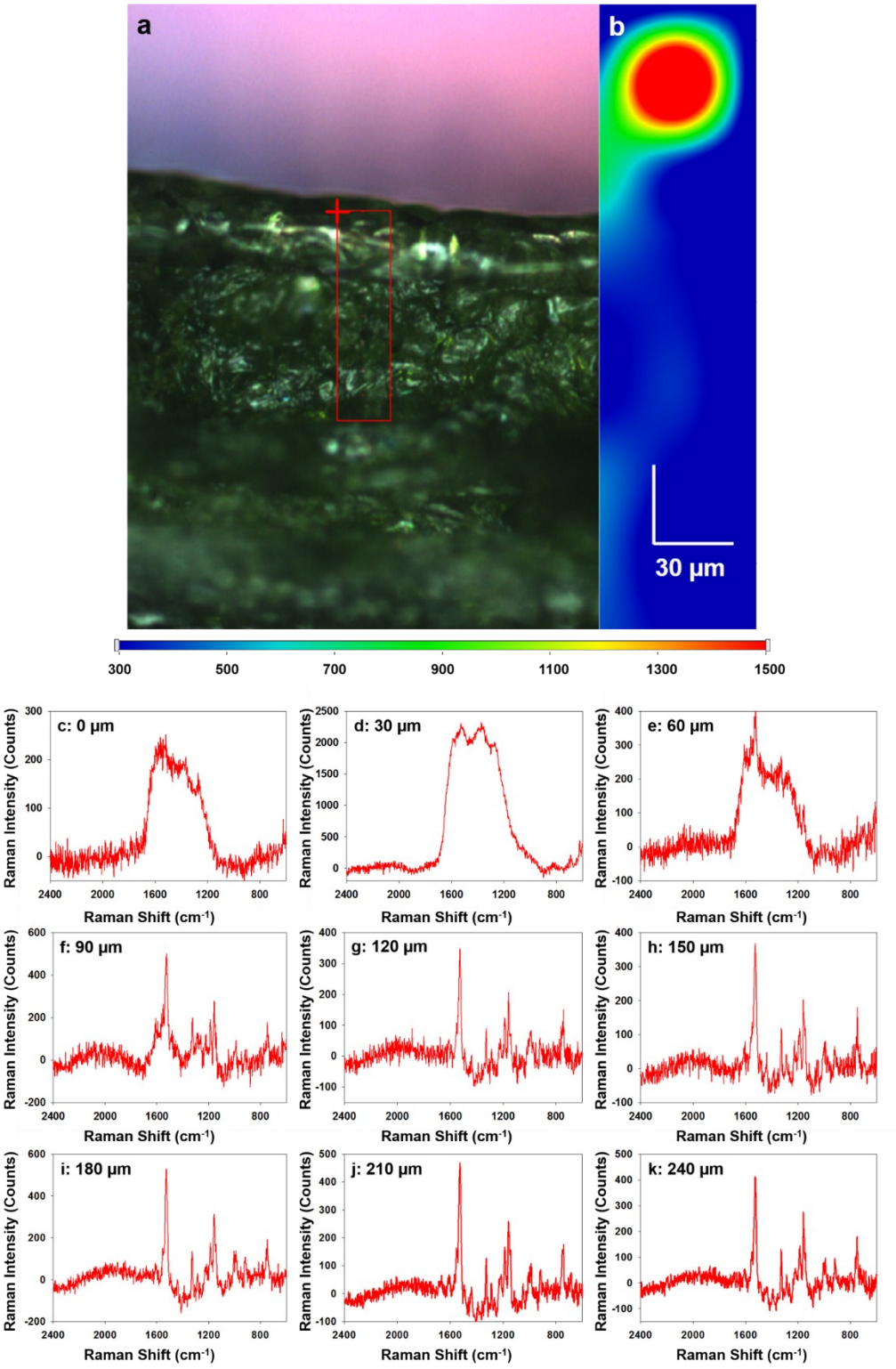

Figure S7. Optical (a) and 2D Raman mapping (b) of the cross section of the spinach leaves deposited with 35 nm Au NPs. (c-k) SERS spectra collected from different depths.