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# 3D Dynamic SERS Imaging of Intracellular Transport Pathways

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## 1. Introduction

Raman spectroscopy has been utilized for material analysis because Raman spectra provide information about molecular or lattice vibration of materials in a sample. Recently, Raman spectroscopy has been combined to laser scanning microscopy to image biological samples with the chemical information that cannot be detected by ordinary fluorescence techniques. However, the efficiency of Raman scattering is very weak, and therefore it is difficult to detect low-concentration molecules taking important roles for cellular functions. We have applied surface-enhanced Raman scattering (SERS), which can be observed at a surface of metal nanostructure, to enhance the detection efficiency of biological molecules participating a cellular events [1][2][3]. We introduced gold nanoparticles with a diameter of 80 nm into a HeLa cell and confirmed the strong Raman scattering from the positions that the particles exist.

## 2. Dynamics SERS imaging

We have built a microscope system that can trace the motion of a nanoparticle in a living cell three dimensionally with simultaneous observations of Raman spectra from the particle to investigate the origin of the strong Raman scattering. We used the motion of gold nanoparticle in a living cell for scanning the cellular interiors by SERS[4]. The gold nanoparticle moves inside the cell through intracellular transportation or diffusion and reports the surrounding molecules as SERS spectra with position accuracy of several tens of nanometers. We observed three dimensional nanoparticle motion and SERS simultaneously every 100 msec by using our custom-built Raman microscope system. Fig.1 shows the time-dependent SERS intensity obtained from a gold nanoparticle in the cytosol of the sample. Fluctuations in Raman intensity and shift, presumably associated with the dynamics of the particle in the cell, were observed.

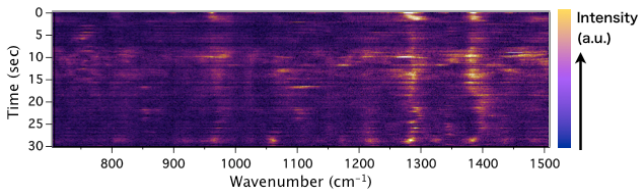


Fig.1 Time-dependent SERS spectrum intensity in the HeLa cell. 100 msec/spectrum

Fig. 2 is a 3D color map of the molecular distribution calculated by mapping the Raman intensity in Fig. 1 with position where the Raman spectrum was detected. The color in each dot shows the Raman peak intensity at 762 $cm^{-1}$ (red), 1069 $cm^{-1}$ (green), and 1390 $cm^{-1}$ (blue), which can be assigned to the vibration mode of ribose phosphate, phosphate ($PO_2^-$), and $COO^-$, respectively. The change in the Raman signal along the trajectory shows that these Raman peaks appeared depending on the position or the motion of the particle. For example, the Raman signal at 1390 $cm^{-1}$ appeared when the particle was going through transportation; while, the Raman signals at 762 $cm^{-1}$ and 1390 $cm^{-1}$ were seen at the start and the end of the transportation, respectively. Hence, these motion- and position-dependent changed of the Raman spectrum reveal information about molecules related to the process of organelle transportation or to the environmental change around the particle during the transportation.

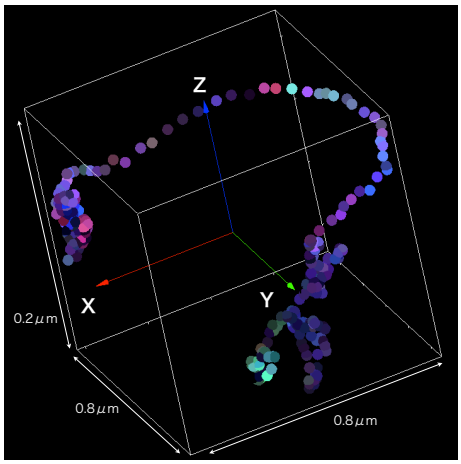


Fig.2 3-dimensional trajectory of the gold nanoparticle and SERS color map. 762 $cm^{-1}$, 1069 $cm^{-1}$, and 1390 $cm^{-1}$ are colored in Red, Green and Blue respectively.

## 3. Conclusions

We have developed a SERS microscope that simultaneously tracked the motion of metallic nanoparticles in three dimensions and acquire SERS spectra for probing cellular interiors. The particle-position and motion-dependent SERS spectrum was observed from the gold nanoparticle transported in the cytosol of a living HeLa cells. The proposed scan-less imaging system could provide molecular information of biological functions responding to exogenous materials such as drugs or viruses with high temporal and spatial resolution.

## References

[1]K. Kneipp,et al., Physical Review Letters, vol. 78, no. 9, pp. 1667–1670, Mar. 1997.
[2]J. Kneipp et al., Journal of Raman Spectroscopy, vol. 40, no. 1, pp. 1–5, Jan. 2009.
[3] K. Fujita et al., Journal of biomedical optics, vol. 14, no. 2, p. 024038, 2010.
[4] J. Ando et al., Nano letter, pp. 5344–5348, 2011.