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Cell Imaging with two-photon induced photoluminescence from gold nanoparticles

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[Introduction] Optical cell imaging has become a powerful tool in the biological and biomedical studies. In this presentation, we introduce a novel optical imaging method using the two-photon induced photoluminescence (TPIPL) from gold nanoparticles. The optical properties of gold nanoparticles under near-field and far-field excitation were systematically investigated with a scanning near-field optical microscope (SNOM) and a two-photon laser scanning microscope (TPLSM). Based on this, the living cell imaging with TPIPL from the gold nanoparticles is demonstrated.

[Experimental] Various sizes of gold spherical colloids (25nm, 50nm and 100nm diameter) were purchased from British Biocell International. The triangular gold nanoplates were synthesized from $HAuCl_4$ with a plant extract as a reductant. The normal baker yeast cells were used as the sample for cell imaging in our experiments. Cultured yeast cells were washed with phosphate buffer solution for several times, and then incubated with gold nanoparticles at room temperature. Therefore some cells were conjugated to the gold nanoparticles. To prepare dried samples, the gold nanoparticle colloid or the yeast cell suspension was diluted and dropped on a coverglass with grid marks, and dried in the air. The samples of living cells were prepared by sandwiching the cell suspension between a slideglass and a coverslip, and the edge was sealed with fingernail polish.

The near-field optical properties of nanoparticles and the cells conjugated to nanoparticles were investigated with a SNOM system. A femtosecond Ti:sapphire laser was used as a light source. Luminescence was excited by femtosecond pulses at 780 nm through a near-field fiber probe, and its spectrum was measured by a polychromator-CCD system. Near-field two-photon excitation image was obtained by detecting the luminescence intensity while scanning the sample. TPIPL from either gold nanoparticles or dried cells conjugated to nanoparticles was observed. Representative results for the cells with gold nanoplates are shown in Figure 1. By comparing the SEM image (Fig. 1a) and the near-field two-photon excitation image, it is clearly seen that the TPIPL emission spots were coming

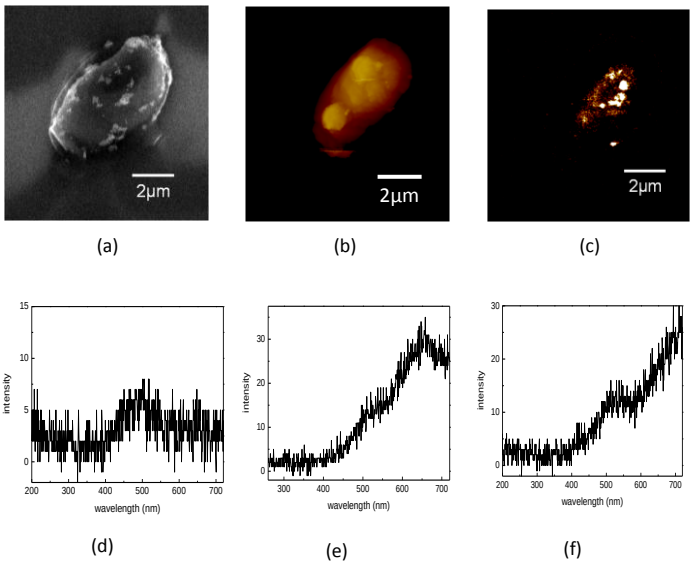

Figure 1. (a) SEM image of a dried yeast cell conjugated to triangular gold nanoplates. The mean base-length of the triangles is about 300nm. (b) Topographic image of the same cell taken by the SNOM apparatus. (c) Near-field two-photon excitation image of the same cell. Excitation: 780 nm. (d) to (f) Typical spectra of TPIPL from the sample. The spectral region longer than 710 nm was cut off by the filter.

from gold nanoplates on the cell surface. The TPIPL spectra measured for three different positions on the cell were shown in Figure 1d to 1f. The features observed are consistent with those observed in previous studies.

The gold nanoparticles dispersed on a glass substrate, or adsorbed on the cell surface were also found TPIPL emissive under far-field excitation by a femtosecond Ti:sapphire laser. Figure 2a shows a TPIPL image taken by a TPLSM for a dried yeast cell conjugated to several gold nanoplates. Each luminescence spot in the TPIPL image was tracked by the corresponding magnified SEM images (Figs. 2c to 2f). It can be known that either a single nanoplate (ex. Fig. 2e), or the aggregates of plates (Figs. 2c, 2d and 2f) could emit strong TPIPL. For spheric particles, however, TPIPL was found only from their aggregates. A SEM image of a couple of dried yeast cells that were fully covered by gold nanoplates, along with some naked cells, is shown in Fig. 3a. A TPIPL image of the same cells is shown in Fig. 3b. Only the cells conjugated to gold nanoplates are visible in the TPIPL image. The TPIPL image of the same cells immersed in water (Fig. 3c) indicates the feasibility of imaging cells in aqueous environment. In fact, we succeeded in observation of living yeast cells conjugated to gold nanoplates in water (Fig. 3d). For comparison, cell imaging with gold nanospheres (diameter 50 nm) instead of nanoplates was tried. The results are shown in Figs. 3e and 3f. TPIPL are visible only where the spheres are aggregated.

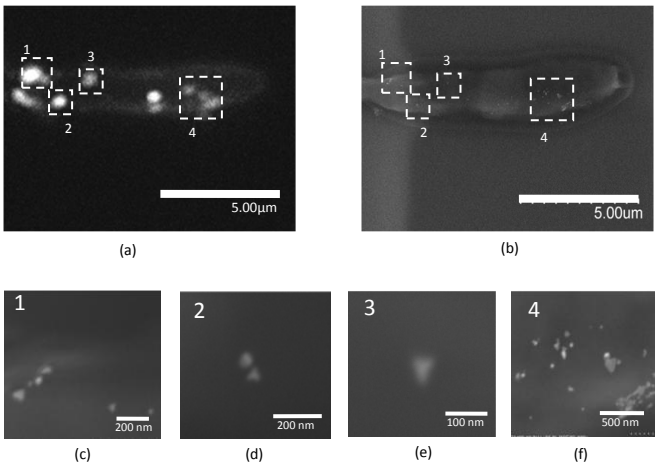

Figure 2. (a) TPIPL image of cells conjugated to triangular gold nanoplates taken by a TPLSM. Incident laser power was 1 mW. The base lengths of the plates were mostly in the range between 70 and 100 nm. (b) SEM image of the same cells. (c) to (f) Enlarged SEM images for the luminescence spots marked in (a) and (b).

[Discussion] The TPIPL spectra observed for the cells conjugated to nanoparticles were essentially consistent with those for nanoplates on glass substrates reported previously. The results indicate that the TPIPL from the nanoparticle-conjugated cells should be attributed to the gold nanoplates adsorbed on the surface of the cell, and was little influenced by the combination with cell. The results of far-field TPLSM studies confirmed the feasibility to image the living cells with TPIPL from nanoparticles. The comparison of imaging with gold nanotriangles and nanospheres indicates merits of labeling cells with gold nanoplates over nanospheres.

In conclusion, we have demonstrated an optical imaging method for living cells using gold nanoparticles, which is expected to be non-photobleaching and nontoxic.

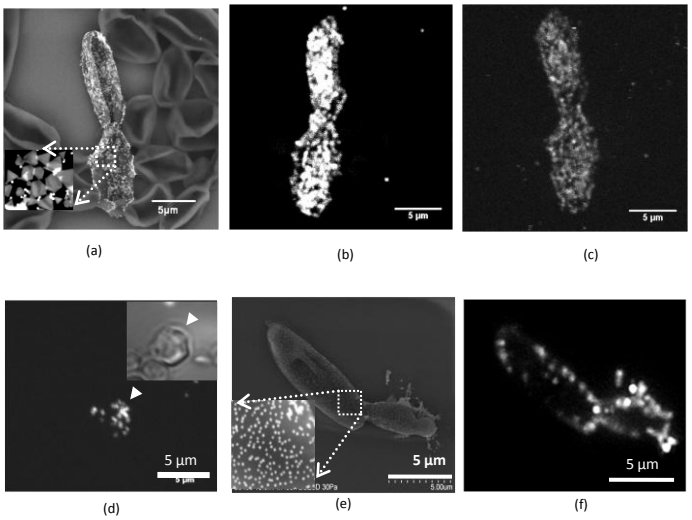

Figure 3. (a) SEM image of dried yeast cells conjugated to triangular gold nanoplates and some naked dried yeast cells. The average base length of nanoplates is ca. 300 nm. (b) TPIPL image of the same dried cells. (c) TPIPL image of the same cells after immersed in water. (d) TPIPL image of a living cell in water. Inset is the corresponding optical transmission microscope image. (e) SEM image of cells conjugated to 50 nm diameter spheric gold particles. (f) TPIPL image corresponding to (e). Incident laser powers for the TPIPL images were 1 mW for (b), (c), and (f), and 2.5mW for (d).