Laser targeted photofabrication of gold nanoparticles inside cells

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Abstract

Nanoparticle manipulation is of increasing interest, since they can report single molecule-level measurements of the cellular environment. Until now, however, intracellular nanoparticle locations have been essentially uncontrollable. Here we show that by infusing gold ion solution, focused laser light-induced photoreduction allows in-situ fabrication of gold nanoparticles at precise locations. The resulting particles are pure gold nanocrystals, distributed throughout the laser focus at sizes ranging from 2 to 20 nm, and remain in place even after removing the gold solution. We demonstrate the spatial control by scanning a laser beam to write characters in gold inside a cell. Plasmonically enhanced molecular signals are then detected from nanoparticles, allowing their use as nano-chemical probes at targeted locations inside the cell, with intracellular molecular feedback. Such light-based control of the intracellular particle generation reaction also offers avenues for in-situ plasmonic device creation in organic targets, and may eventually link optical and electron microscopy.

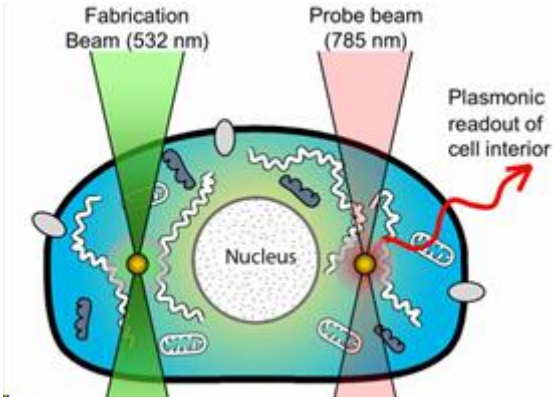

Figure 1 Photoreduction of aqueous gold solution by targeted laser beam.

While nanoparticles do not freely pass through a cell, the cell is permeable to gold ion solution. By infusing the cell culture dish with gold ion solution, focused 532 nm irradiation can then trigger photoreduction at an area of interest and ionic gold can later be washed out, leaving permanent fabricated gold nanoparticles at the laser focus. Intracellular spectra can then be studied by Raman spectroscopy, without luminescent interference, using near-infrared light excitation (785 nm).

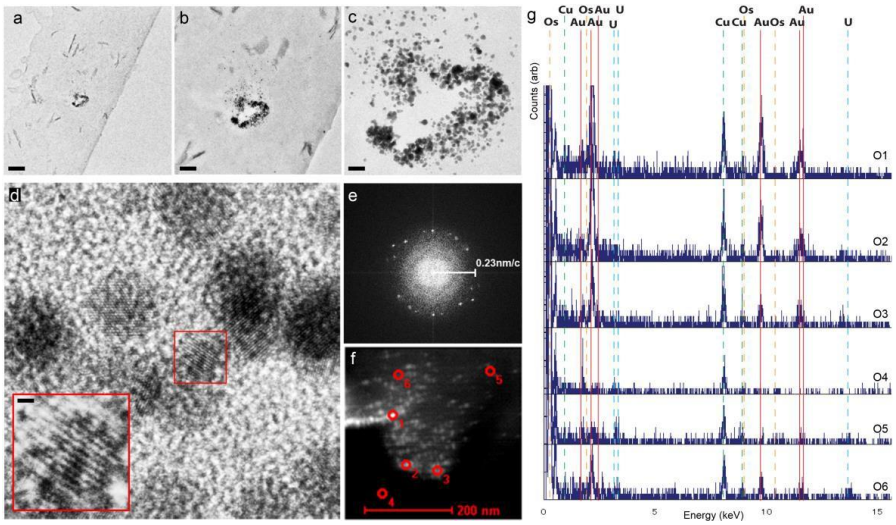

Figure 2 Electron microscopy images and X-ray dispersive analysis of fabricated particles. (a-c) show the laser focus region with aggregation of nanoparticles. (d) shows a high resolution image of single particles. The gold crystal lattice is visible (see enlarged inset). The lattice spacings are measured by spatial frequencies in the Fourier transform shown of (d), shown in (e), and corresponds to the 2.35 Å interplanar spacing of gold. (f) shows scanning transmission electron microscopy, with X-ray dispersive analysis in (g) confirming the signature of gold with Au peaks marked in red. Other heavy metals appear from the sample preparation (osmium, uranium) and copper appears from the substrate. Together, these results show that the particles fabricated by laser range in size from 2 to 20 nm and are composed of pure gold crystals, based on the X-ray analysis and lack of inclusions observed in the lattices. The distribution of nanoparticles in (b) appears to be confined to a region of approximately 340 nm in diameter. Scale bars for (a-c) are 310, 136, and 39 nm, respectively, while the inset scale bar for (d) is 0.54 nm.

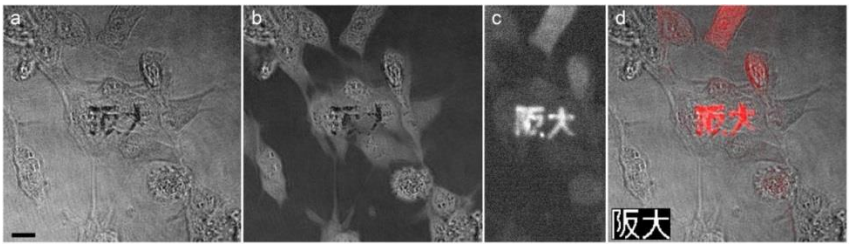

Figure 3 Phase and spectroscopic imaging of photofabricated characters in gold inside cells. Absorption imaging (a) and phase imaging (b) by digital holographic microscopy (DHM) show the fabricated pattern to some degree, but the luminescence image taken via an imaging spectrometer (c) shows the pattern most clearly. An overlay (d) shows luminescence in the red channel on top of the absorption image (greyscale), with the small inset showing the actual scan pattern for the gold fabrication. The results show that locations of interest can be targeted for photofabrication. The scale bar is 12 μm.

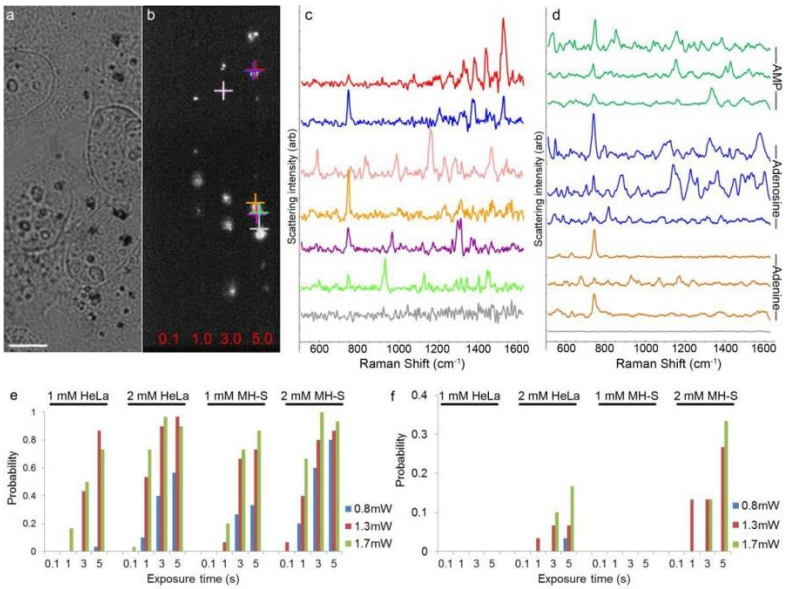

Figure 4 Surface enhanced Raman scattering from fabricated regions. While small nanoparticles do not usually provide a basis for surface-enhanced Raman measurements, point irradiation of 532 nm, 5 mW laser light for 0.1 to 5 produced regions shown in brightfield (a) and luminescence (b) where exposure times for each column of points are also denoted in (b) in red. The measurement of SERS signals was done by 785 nm light, where SERS data were automatically detected (see methods) to avoid bias. SERS was only detected from regions where 532 nm light was irradiated. SERS-generating locations are marked in (b) by +, with each corresponding spectra shown in (c). SERS spectra of AMP, Adenosine and Adenine (3 representative spectra for each) were also measured for comparison (d, see Methods for details). The horizontal axis shows Raman scattering shift in cm^{-1}. Probabilities for the occurrence of luminescence and SERS from fabricated regions are shown in (e) and (f), respectively, for varying laser power, exposure time, cell type, and HAuCl4 concentration (1 or 2 mM). Error bars are not shown; probabilities are number of occurences divided by number of trials n where for HeLa cells, n=30 and for MH-S macrophages, n=15. The scale bar is 10 μm.