

Optical Injection of Gold Nanoparticles into Living Cells

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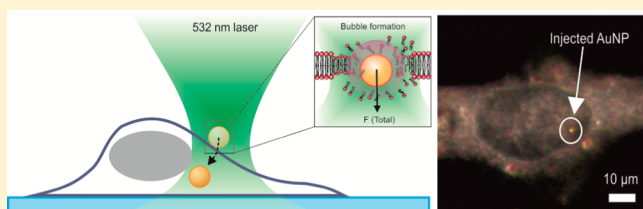
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S Supporting Information

ABSTRACT: The controlled injection of nanoscopic objects into living cells with light offers promising prospects for the development of novel molecular delivery strategies or intracellular biosensor applications. Here, we show that single gold nanoparticles from solution can be patterned on the surface of living cells with a continuous wave laser beam. In a second step, we demonstrate how the same particles can then be injected into the cells through a combination of plasmonic heating and optical force. We find that short exposure times are sufficient to perforate the cell membrane and inject the particles into cells with a survival rate of >70%.

KEYWORDS: Nanoparticles, optical force, plasmonic heating, nanoinjection



Nanoparticles provide numerous advancements for contemporary personalized medicine that aspire both diagnostics and treatment of a disease in one single system.^{1,2} While there has been many reports on nanotheranostic applications of nanoparticles inside living cells,^{3,4} the major challenge remains to find proper means of delivering particles one-by-one to the cell interior and to do this also with full spatiotemporal control.

The cell membrane is almost impermeable for large molecules or ions, which makes the active nanoparticle delivery across the membrane barrier difficult. Biochemical strategies mostly employ carrier molecules such as dendrimers,⁵ liposomes,⁶ or polymers⁷ for cell transfection, but there are also a number of methods to overcome the membrane barrier by physical means such as electroporation,⁸ sonoporation,⁹ and optical approaches using laser light.^{10,11} For the latter, living cells are typically exposed to a strongly focused, high energy, nano- to femtosecond laser pulse, which generates a transient hole in the cell membrane. The hole then remains open for a short time period to allow small molecules such as nucleic acids,^{12–14} drugs,¹⁵ or even nanoparticles to passively diffuse into the cells.¹⁶ Such laser-assisted photoporation has certain advantages over other methods for being contactless (and therefore sterile), efficient, and specific (cells can be singled out from a whole cell population).

Efficient generation of membrane pores, however, requires laser pulses with very high peak intensities that bear the risk of photodamage and therefore impose a potential threat to cell viability. Here, gold nanoparticles can provide a solution for establishing a less harmful approach for light-assisted nano-injection by taking advantage of their plasmonic properties.

Gold nanoparticles absorb light very efficiently at the particle plasmon resonance that leads to a strong local temperature rise within picoseconds upon irradiation with laser light.^{17,18} This

strong increase of temperature on a nanoscopic area can in turn lead to an increase of membrane permeability^{19,20} or the formation of nanobubbles that results in a mechanical disruption of the cell membrane and the formation of pores.²¹ Both effects can consequently be applied for photoporation of cell membranes via localized heating^{22,23} or laser-mediated cavitation^{24,25} and have successfully been used for laser-induced gene transfection of eukaryotic cells.^{26–28}

Gold nanoparticles, however, are at the same time also subject to optical forces when they are irradiated with a focused laser beam.^{29,30} In other words, a gold nanoparticle can be pushed and heated simultaneously. This combined properties can be used to inject gold nanoparticles into lipid vesicles (GUVs) as shown previously³¹ but to date it has not been possible to inject single nanoparticles directly into living cells.

In this report, we demonstrate how individual gold nanoparticles can be patterned with high control and precision on the surface of a living cell and how, in a second step, they can be injected, one-by-one, into the cell by using a combination of optical forces and plasmonic heating.

A schematic of the experiment is shown in Figure 1. An upright darkfield microscope (Figure 1a) was used for imaging the cells and individual (80 nm) gold nanoparticles. A continuous wave laser ($\lambda = 532$ nm) was coupled into the microscope and focused on the sample. The injection of individual particles inside the cells is accomplished in a two-step process. First, gold nanoparticles are printed on the cell membrane by optical force (Figure 1b). The laser is focused on the glass substrate underneath a cell. Gold particles in solution

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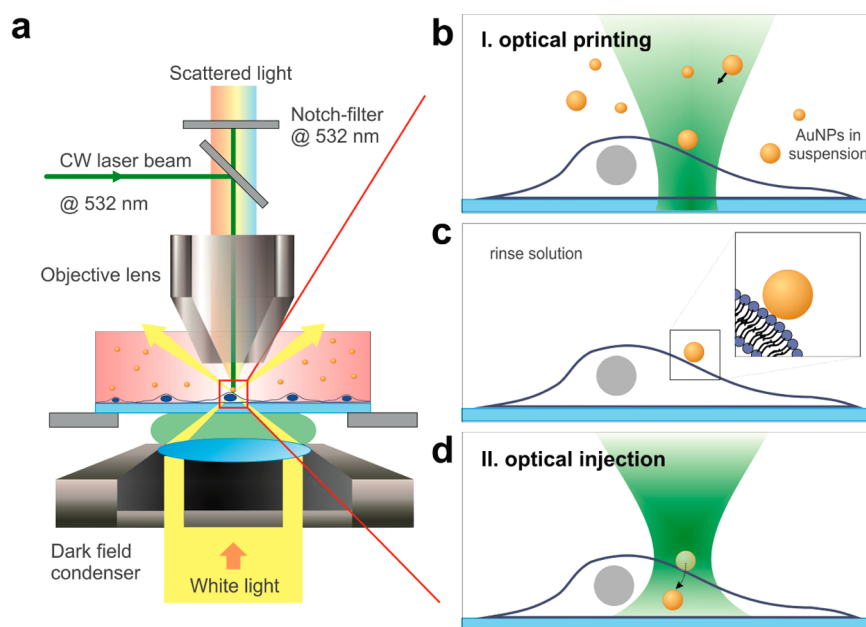


Figure 1. Printing and injection of gold nanoparticles into living cells. (a) Schematic of the experimental setup: CHO cells are cultured on a Petri dish and gold particles are added to the cell medium. A laser beam is coupled into the darkfield microscope for particle manipulation. (b) First, the laser beam is focused to the glass substrate, which results in a defocused laser spot on the cell membrane. Gold nanoparticles entering the laser beam are pushed toward the beam center along the light propagation axis and printed on the cell surface. (c) After the printing, the cell culture medium is exchanged to wash away any particles in solution. (d) For nanoparticle injection, the laser beam is focused directly onto individual gold particles to induce the highest amount of heat and force in order to push the particles directly into the cell.

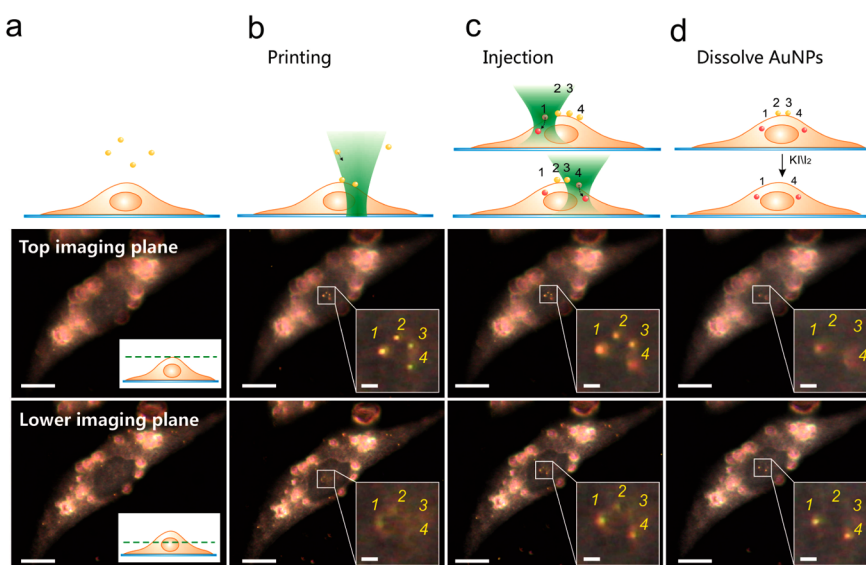


Figure 2. Darkfield images for laser printing and injection of gold nanoparticles into a cell. (a) CHO cells growing on glass substrate were mixed with 80 nm particles and the cells were imaged at two different focal planes. (b) A laser beam was focused on the glass substrate underneath the cell to print four particles (Nr. 1 to 4) to the cell surface. (c) Nanoparticle injection was achieved by focusing the laser directly onto single selected particles (Nr. 1 and Nr. 4) for 1 s at a laser power of 5 mW. (d) Gold particles attached to the cell membrane (Nr. 2 and Nr. 3) are dissolved upon addition of KI/I₂, while the particles that were injected into cell (Nr. 1 and Nr. 4) are preserved. (Scale bars: 10 μm , insets: 1 μm .)

that are entering the laser beam are pushed toward the substrate and then positioned on the cell by a forward directed scattering force (Figure 1b). By moving the laser to different areas, it is possible to print nanoparticles one-by-one onto specific targeted sites on the cell surface (please see Supporting Information, Figure S1 for details). After the printing, the medium is rinsed to remove excessive gold particles (Figure 1c). Finally, injection occurs when the laser beam is focused directly onto single particles that are attached to the cell

membrane to achieve the strongest increase of optical force and temperature (Figure 1d).

The result of the complete injection process for a single cell is shown in Figure 2. Chinese hamster ovary (CHO) cells were grown in a glass culture dish at the confluence of 60–80%. Gold nanoparticles with a diameter of 80 nm were added to the cell culture medium. The particles were stabilized with a citrate coating to avoid any unspecific attachment to the cells (Figure 2a). Four gold nanoparticles were printed next to each other

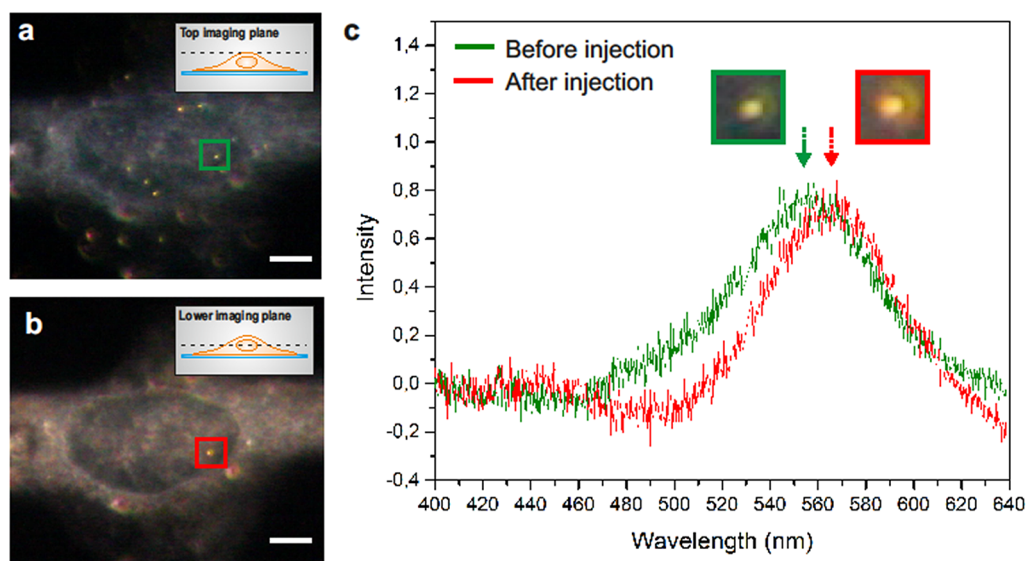


Figure 3. Rayleigh scattering spectroscopy of a gold nanoparticle before (a) and after (b) laser injection. (c) The spectra showed a red shift of approximately 10 nm following laser injection which corresponds to the change of the refractive index from 1.33 to 1.38 from the outside to the inside of the cell. (Scale bar: 10 μm .)

onto the surface of a single cell (Figure 2b). Then, two of the nanoparticles (Figure 2c, Nr. 1 and Nr. 4) were injected into the cell by focusing the laser directly onto them. The successful injection was immediately visible under the microscope because the particles were pushed out of focus and to a lower focal plane in the cell center (Figure 2c).

We applied a method reported by Xia et al.³² to further prove that the gold nanoparticles were actually inside the cell. For this approach, KI/I_2 is used as an etching reagent to selectively dissolve any gold nanoparticles on the cell surface, while those that are inside the cell are protected and not exposed to the chemical reagent (Supporting Information, Figure S2). It is important to note that the KI/I_2 etchant is of low toxicity to cells when used below 0.34 mM (for I_2). A cell viability of over 90% was confirmed even after an etchant exposure for over 5 min, which was in good agreement to previous reports.³²

The progress of the etching was directly observed under the darkfield microscope. The gold nanoparticles that were sitting on the cell membrane (Figure 2d, Nr. 2 and Nr. 3) were dissolved within 20 s upon addition of the reagent while those inside the cells were unharmed (Figure 2d, Nr. 1 and Nr. 4).

The successful injection of the gold nanoparticles was also confirmed by Rayleigh scattering spectroscopy as shown in Figure 3. A single gold nanoparticle was laser printed on the cell and a scattering spectrum was recorded (Figure 3a). The same particle was then injected into the cell and the scattering spectrum of the particle was recorded again (Figure 3b). The comparison of both spectra showed that the resonance peak was shifted from 555 to 565 nm before and after injection (Figure 3c). The peak shift of ~ 10 nm agrees well to what is expected due to the refractive index change from the outside ($n \sim 1.33$) to the inside ($n \sim 1.38$) of the cell.^{33,34}

We performed numerical calculations to estimate the amount of heat and force induced by the laser beam for both the printing and injection step of the experiment to reveal the nature of the injection mechanism. The force maps of the calculated total optical forces exerted on gold nanoparticles for different laser powers are shown in Figure 4a–c. Experimentally, we found that a laser power of at least 5 mW was

necessary to print particles successfully and reproducibly to the cell surface that corresponds to a total force in the piconewton range. In general, higher laser powers lead to stronger optical forces but they are also leading to stronger heating. During printing, the heating of the particle was not significant, because the beam is focused to the surface of the underlying substrate and the nanoparticle therefore never enters the center of the beam where the light intensity is the highest. For the injection, however, the laser is directly focused on the particles, which leads to a strong and instantaneous rise of the nanoparticle's temperature. A schematic illustration of the suggested nanoparticle injection mechanism is shown in Figure 4d. At a certain point, the heating on the nanoscale can lead to nanobubble formation, namely if the temperatures go above the spinodal decomposition temperature of water at around 320 $^\circ\text{C}$.³⁵ Indeed, we observed the formation of nanobubbles at the laser powers that were used for the injection process for both, particles sitting on a cell membrane and on a glass substrate, respectively (please see Supporting Information, Figure S3). The expansion and collapse of these bubbles is accompanied by the production of a shock wave that causes the rupture of the cell membrane.³⁶ At this point, the scattering forces exerted on the nanoparticle lead to its injection into the cell through a membrane "hole".

We found that nanobubbles were always formed for a laser power of 5 mW (which corresponds to a laser power density of ~ 1185 kW/cm^2) where surface temperature of the gold particles is well above 350 $^\circ\text{C}$ (Figure 4e). For comparison, no bubble formation and also no nanoparticle injection was observed for a laser power of 1 mW (laser power density ~ 237 kW/cm^2) where the temperature of the gold particle is only around 112 $^\circ\text{C}$.

The formation of nanobubbles is not only instrumental for the perforation of the membrane, but also aids cell survival for the reason that the very high particle temperatures that are reached are likely hazardous to the cells. This is particularly true for heating under CW illumination as the temperature profile around the particle expands into the surrounding as a function of $1/r$.¹⁸ The steam temperature of the nanobubble, however,

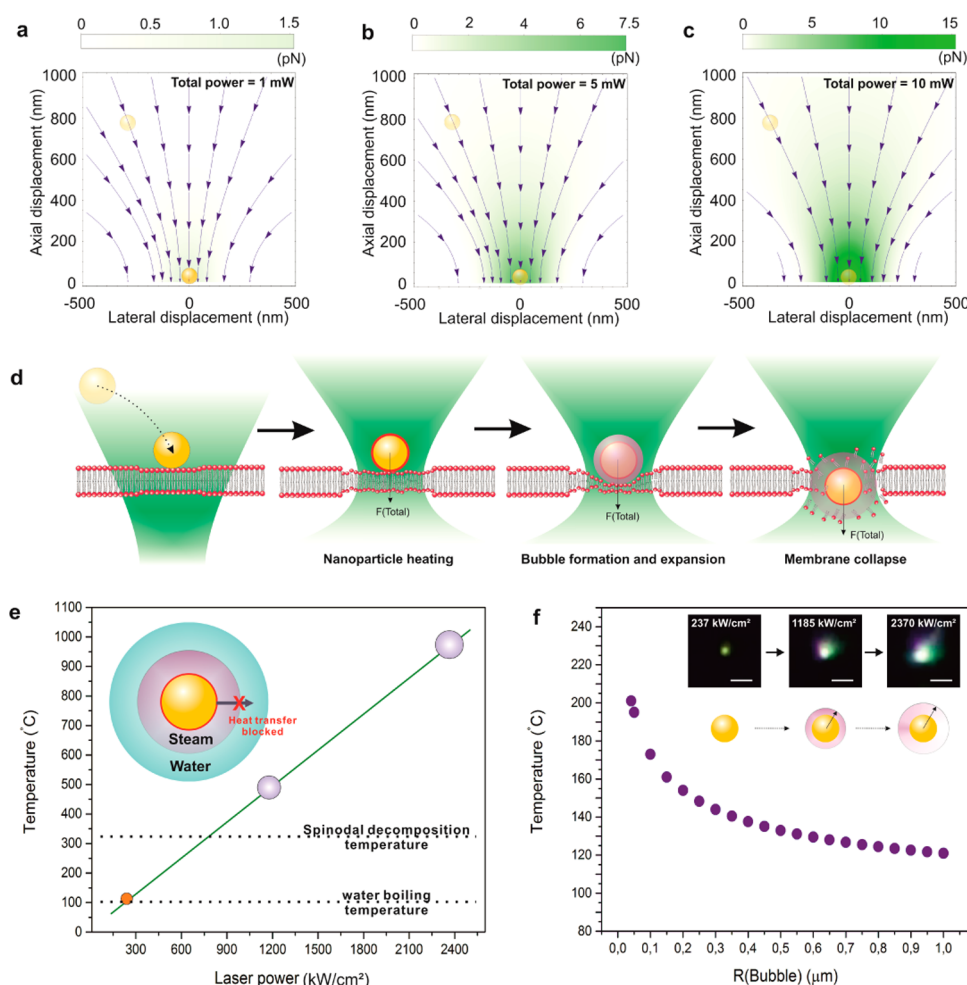


Figure 4. (a–c) Calculated optical forces exerted on 80 nm gold nanoparticles upon exposure with a Gaussian laser beam ($\lambda = 532$ nm). The color indicates the magnitude of the total force. The arrows illustrate the direction of the force that is guiding the particle along the optical axis and toward the cell membrane. (d) Schematic representation of the injection process: exposure of a gold particle with a focused laser beam leads to a rapid temperature increase and nanobubble formation around particle. The expansion and collapse of bubble leads to membrane rupture and the formation of a transient hole. The nanoparticle is then immediately pushed into the cell by the optical force. (e) Calculated NP temperature as a function of laser power. Nanobubble formation was experimentally observed for a particle temperature above 350 °C (Supporting Information, Figure S3). The steam shell surrounding the particles works as an insulating barrier that shields the cell from the hyperthermal damage. (f) Calculated steam temperature as a function of the nanobubble size.³⁷ Figure inset: Examples of nanobubbles formed after exposure of single gold nanoparticle with a focused green laser beam. The bubble size was found to be increasing for higher laser powers. (Scale bar 500 nm.)

stays moderate in comparison and the vapor shell that furthermore provides a shield against further heat transfer into the medium (Figure 4e).

We followed the model of Fang et al.³⁷ to estimate the steam temperature as a function of the nanobubble size (Figure 4f). The system is assumed to reach a steady state once the bubble is formed around the gold nanoparticle and the Laplace pressure is balanced by the vapor pressure of the steam. The relation between the nanobubble size and the steam temperature can be estimated by $P_0 + ((2\gamma(T_s))/R_B) = C e^{(H/(k_B T_s))}$, with T_s being the temperature of saturated steam, R_B is the bubble size, P_0 is the atmospheric pressure, γ is the surface tension coefficient that is the function of T_s , C is a constant, k_B is the Boltzmann constant, and H is the enthalpy of water evaporation.³⁷ As shown in Figure 4f, the total temperature of the steam does not reach temperatures higher than 200 °C.

We investigated the effect of nanoparticle injection on cell survival as shown in Figure 5. Cell viability was confirmed by the enzymatic conversion of nonfluorescent cell-permeant calcein AM to the intensely green fluorescent calcein by

ubiquitous intracellular esterase inside living cells. On the other hand, dead cells are identified by the bright, red fluorescence of Ethidium Homodimer-1 (EthD-1), which can enter cells only if the plasma membrane is damaged and becomes fluorescent upon binding to nucleic acids. After the printing step, a cell viability of ~64% was observed when using a laser power of 5 mW as shown in Figure 5c. The viability significantly dropped for higher laser intensities and was only 30% for a laser power of 25 mW.

For the injection step (Figure 5 b,d), the application of a 25 mW led to a cell death rate of over 50%, while both 10 and 5 mW resulted in relatively high viability rates of 73% respectively. Notably, although the surface temperature of gold nanoparticle when being injected was beyond the normal biological tolerance, the insulating effect caused by the vapor shell protected most of the cells from being killed. The irradiated gold nanoparticle thereby caused only a local damage to the cell membrane, rather than thermal degradation of the whole cell. The results of the cell viability tests furthermore suggest, in accordance with previous reports by other groups,³⁸

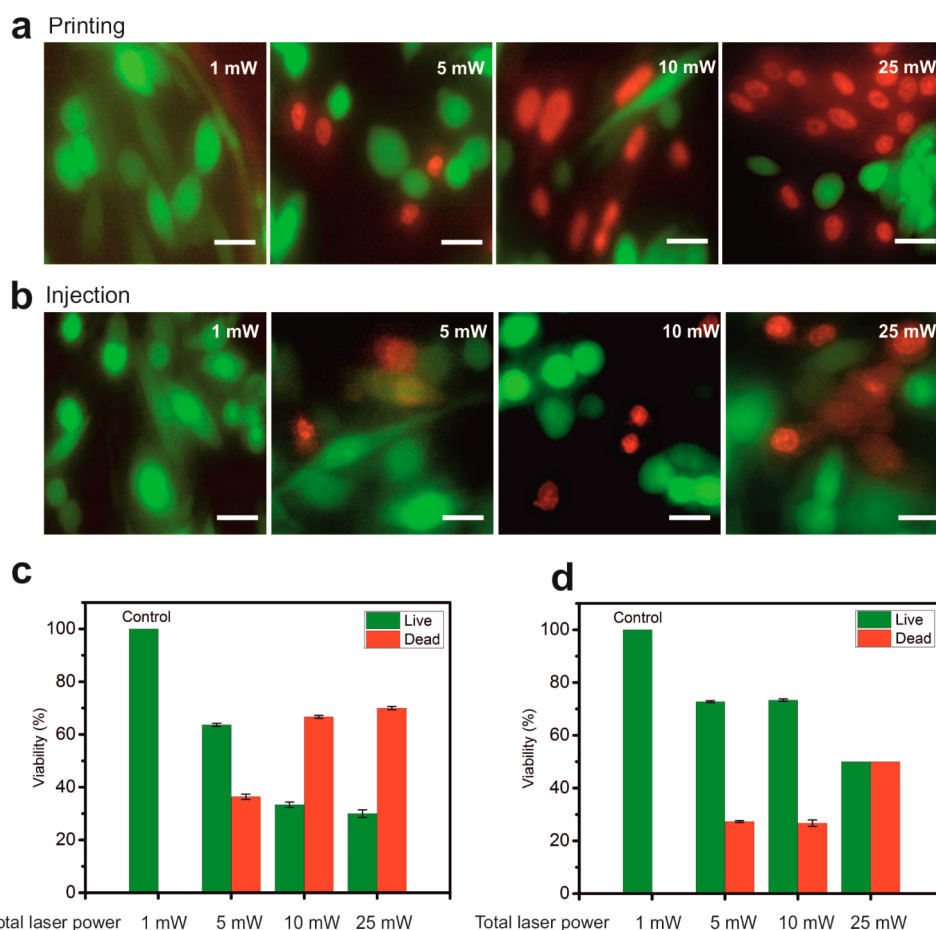


Figure 5. Cell viability tests after laser printing (a) and injection (b) of gold nanoparticles with different laser powers. Living cells appear green while dead cells appear red in epifluorescence (scale bar 40 μm). The survival rate of cells after printing (c) and injection (d) were measured independently for both processes. In all cases, the percentages of living cells were found to be decreasing for higher laser powers.

that the holes that were generated in the cell membrane were also quickly closed within a few minutes and that the cell-impermeant EthD-1 dye could therefore not enter the cell, already shortly after laser injection.

In summary, we have demonstrated an all-optical approach to deliver 80 nm gold nanoparticles into a living mammalian cell. This method relies only on a combination of optical forces and plasmonic heating of single gold nanoparticles with a focused laser beam. The formation of nanobubbles was found to be instrumental to overcome the membrane barrier and push the particles into the cells. Overall, our results illustrate how nanoscopic objects can be actively delivered into living cells using light, which paves the way for future biomedical applications in nanotheranostics and drug delivery.

■ ASSOCIATED CONTENT

● Supporting Information

Materials and Methods section describing the experimental procedures and instrument setups and supporting Figures S1–3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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