Laser tweezers and syringes for cell biology

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Using light to inject a single nanosized biosensor into a mammalian cell enables analysis of its biochemistry.

The mammalian cell is a beautifully orchestrated nanomachine, but like all organized systems sometimes things go wrong. Key to its inner workings are proteins of 1–100nm across. If one of these fails to do its job, diseases ranging from cancer to Alzheimer’s can result, yet understanding protein function by mapping their presence in space and time remains a challenge. Raman spectroscopy permits assessment of local chemistry by shining a laser onto the sample and measuring changes in the light’s properties. A small metallic object may be used to improve the signal strength. This technique, known as surface-enhanced Raman spectroscopy (SERS), can identify cancerous or virally infected cells, and may be key to future disease diagnoses.

Previous SERS studies have used the cell’s own natural ingesting ability to internalize nanoparticles. However, this often results in spectroscopic analysis of the lysosome, the membrane that forms around the sensor after internalization, but not of other, more interesting regions. The ability to inject single nanosized biosensors at will would be a significant step towards controlled spectroscopic analysis of important cellular structures such as the cytoplasm or nucleoplasm, where much of the cell mechanics takes place.

It has become routine to manipulate micro- and nanoscopic objects outside a cell, and larger (micron-sized) objects have been placed in plant cells or highly stressed mammalian cells. Optical insertion of, e.g., fluorophores, chemicals that selectively bind to certain aspects of biochemical machinery and fluoresce proportionally to their activity, has been widely demonstrated, but tweezing and injecting one particle at a time has rarely been attempted. We are the first team to have optically tweezed and injected a single nanoscale particle into a mammalian cell. We have achieved this using two carefully aligned, highly focused laser sources, one to manipulate the nanoparticle and the other to inject it into a cell.

Single-beam gradient-force optical traps, commonly known as optical tweezers, consist of a laser focused to a tiny spot by a high-powered (for example, 100×) aperture-microscope lens. Using optical tweezers, micron-sized particles such as 0.5–10µmdiameter silica or polystyrene spheres can be captured and physically manipulated in 3D. Smaller nanosized objects, such as 50–200nm-diameter gold or silver particles may also be physically controlled, provided the system is carefully aligned. We co-aligned two highly focused lasers, a 1064nm continuous-wave source and an 800nm femtosecond-pulsed one, for tweezing and injecting single 100nm gold particles, respectively.

Our experimental setup is shown in Figure 1. A mercury lamp and filter combination was used to illuminate the sample, allowing visualization of the nanoparticles. The two co-aligned beams

Figure 1. The combined optical tweezers and optical-injection setup. Not to scale. Kohler illumination is a method of providing optimal illumination of the sample. (© Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

Continued on next page
were directed via a relay telescope and dielectric mirror into a 100× Nikon objective. A CCD camera and Köhler illumination, a method of providing optimal illumination, provided visualization of the cells. A floating coverslip separated the cell sample from the toxic immersion oil used for the objective lens. Key to our success was the use of an oil with a high refractive index, not commonly used in imaging microscopy. In optical tweezing, it allows much greater control in manipulating nanoparticles by minimizing spherical aberrations. This means that for any given power the trapping strength is increased, and a further advantage is therefore that the tweezed particle will also heat less as a lower power may be employed.

Other crucial features of our setup were the very powerful 1.4 numerical-aperture 100× objective, the circular polarization of the tweezing beam, and that the system was very well aligned. We achieved this by tweezing 1μm silica spheres with both beams and adjusting until no displacement occurred when toggling between the two beams. As a second check, we tweezed single gold particles onto a coverslip using the 1064nm beam. The 800nm beam was then switched on and a bright backscattered signal was observed from the gold alignment was considered optimal.

For the experiment, we added a dilute suspension of gold nanoparticles to Chinese-hamster ovary cells. We used optical tweezers to place a single 100nm piece of gold on the top surface of a single cell. We used a 40ms dose of the injection beam to open a small transient photopore on the plasma membrane immediately below the particle and used brightfield imaging to see the gold fleck moving downwards during the injection step. Finally, we used a combination of confocal-laser-scanning microscopy and confocal-laser-scanning reflectance microscopy to verify that the nanoparticle was inside the cell by visualizing the position of the cell and particle, respectively. Figure 2 shows a typical result, with a red particle that has been tweezed and injected into the nuclear region of a green cell. The red signal is from reflectance microscopy, and green is from the fluorescent dye FM 4-64, which stains both the plasma membrane and cytoplasm of the cell.

The cell remains an amazingly complicated and interesting device. We believe that using light as a tweezer and to inject single nanobiosensors will provide a hitherto unknown level of control for the cell biologist in mapping intracellular biochemical events. Future studies will focus on tweezing internalized nanoparticles for the purposes of SERS. For a cell near you, the future is bright.

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