PROBE MICROSCOPY

Scanning below the cell surface

Conventional atomic force microscopy probes only the surface of specimens. A related technique called scanning near-field ultrasonic holography can now image nanoparticles buried below the surfaces of cells, which could prove useful in nanotoxicology.

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More and more nanoparticles are used in advanced materials, medical imaging and drug delivery, and their industrial scale production is just around the corner. The flip side of this is the risk associated with human exposure to nanoparticles, whether through unintentional inhalation or deliberate injection and ingestion. Detailed toxicity studies are needed to ensure the safe and successful use of nanoparticles, but numerous challenges exist[1]. In particular, visualizing the way that nanoparticles enter cells is an area that deserves attention.

On page 501 of this issue, Ali Passian and colleagues at the Oak Ridge National Laboratory, the University of Tennessee and Northwestern University report striking images of nanoparticles inside cells obtained by purely mechanical means[2]. Their method does not require using any external labels such as fluorescent or radioactive molecules to tag the cells or particles. This makes the approach simple and promises throughput with minimal sample preparation. Considering the large variety of nanoparticles and cells that exist, these attributes will be immensely useful in determining the fate of nanoparticles that come into contact with biological organisms.

The use of optical microscopes to image particles much smaller than the wavelength of light has proven to be difficult, but it is possible. Diffraction of light waves results in blurry images that have little contrast for objects smaller than the wavelength of light. A clever method to overcome the diffraction limit is to bring the optical sensor close to the sample. If the distance between the sensor and the sample is smaller than the wavelength of light then high resolution images can be generated. This powerful imaging method is generally termed as near-field scanning optical microscopy[3]. The advantages of near-field optical microscopy are balanced by the geometrical constraints on the sample. Unfortunately, objects that are buried several micrometres below the surface, such as nanoparticles that are inside cells, cannot take advantage of near-field optical microscopy.

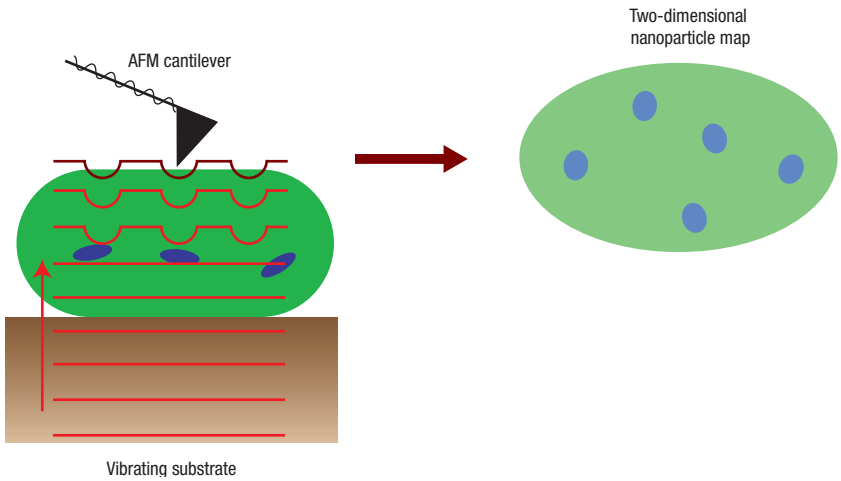

Figure 1 Seeing inside cells with mechanical vibrations. When cells (green) are placed on a vibrating substrate, mechanical vibrations (red lines) passing through the cells are disturbed by nanoparticles inside the cells (blue). By scanning an atomic force microscope over the surface of the cell and detecting these vibrations, it is possible to generate a two-dimensional image that shows the location of the nanoparticles inside the cell (right).

The analogy between ultrasonic waves and light waves is used in the study by Passian and co-workers. Ultrasonic waves are high frequency mechanical vibrations and, like optical waves, they can be used to generate images of samples including cells[4]. The concept of near-field imaging is also applicable to ultrasonic waves[5], but with a significant advantage. The ultrasonic wavelengths used for imaging can be as long as a millimetre, which is much longer than the wavelength of visible light. Nanoparticles that are below the cell surface are still in the ultrasonic near field, so high-resolution images can be generated by near-field techniques.

Passian and colleagues applied this technique, known as scanning near-field ultrasonic holography[6], to image nanoparticles inside cells. In the experiments, they aspirated mice with single-walled carbon nanohorn particles and isolated the macrophages (cells that engulf foreign material) from the lungs and red blood cells for imaging. The cells were placed on a substrate that was vibrated at ultrasonic frequencies of around 4 MHz (Fig.1). As the vibrations propagated through the cells, depending on the objects in their paths, different regions on the wave fronts accumulated different delays or phase shifts. Hence, the original wave front is disturbed after interacting with the nanoparticles. A frequency of 4 MHz corresponds to a wavelength of around a quarter of a millimetre, which is more than a thousand times longer than the dimensions of nanoparticles. This means that the phase shifts due to nanoparticles can be extremely small. Therefore a sensitive detection mechanism is needed.

The team uses the sharp tip of an atomic force microscope to measure the disturbances or delays in the wave fronts by vibrating the microscope cantilever at a slightly different frequency compared with the vibrations emanating from the substrate (much like the way an FM radio receiver converts high frequency electromagnetic waves into much lower

audible frequencies). The two vibrations mix together on the cantilever and create a third vibration at a much lower frequency that is exactly equal to the frequency difference between the two. Any delay in the vibrations through the cell due to the presence of the nanoparticles shows up as a phase shift on the low frequency vibration. Recording this phase shift as a function of position yields the intracellular nanoparticle map of the cell.

The study found nanoparticles (~70–110 nm) inside the lung macrophages and peripheral blood samples, indicating that nanoparticles rather than larger aggregates were taken up by the cells. Whereas the atomic force microscope topography images resolved nanoparticles on the surface of the cell, the ultrasonic phase images revealed a surprisingly large number of additional particles inside the cell at the same location. All of this was further verified by Raman spectroscopy. Measurements with silica nanoparticles confirmed that this technique is able to resolve materials of different stiffness. The results suggest that, by visual inspection of cells, new high throughput *in vitro* screening assays can be developed for studying nanoparticle–cell interactions, the route of uptake into cells and the exposure risk of nanoparticles.

The present technique is sufficient for mapping nanoparticles inside cells, but using higher frequency ultrasonic vibrations or excitation at multiple frequencies together with broadband force sensors[7] may improve contrast and possibly the ability to determine the vertical position of the nanoparticles. With further improvements in spatial resolution, modelling, and quantification, it should also be possible to determine the effect of nanoparticle size and shape on their cellular uptake. The new method for imaging nanoparticles below the cell will surely generate much excitement and help speed up studies in nanotoxicology.

References

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