High-Resolution 3D Imaging for Nanoparticle and Cell Systems

Cost-effective and accurate imaging methods are needed to support studies of nanoparticles in cell systems. This need spans a scale from a few nanometers to many tens of micrometers and may include living tissue. The capabilities of conventional optical microscopy combined with recent advances in desktop computing power, were applied to nanoparticle location in human cells, ultimately to aid in the selection and development of the most suitable imaging methods.

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Nanoparticle and cell systems can be studied using strongly fluorescent nanoparticles called quantum dots, typically a few nanometers in size. The fluorescence emission of quantum dots is readily detected by optical microscopy, however the ability to locate quantum dots to a precision below the diffraction limit of light is a problem. Resolution limitations can be approached by other methods such as transmission electron microscopy (TEM), but with trade-offs. Specialized approaches such as optical super-resolution are in development.

A variety of methods may be employed in a complementary fashion to try to address all imaging needs ranging from the nanoscale to the scale of relatively large organic systems of many tens of micrometers. Factors involved in the decision point for selecting conventional optical, specialized optical, or electron beam methods for nanotechnology research and development in general, have bearing on more than just the basic imaging capabilities. These may include instrumentation costs, the extent to which live-cell system experiments are possible, which imaging artifacts may be present, whether or not chemical measurements can be included, speed, and other factors.

The ability to locate fluorescent quantum dots by optical microscopy to a precision below the diffraction limit of light is difficult. NIST scientists are developing specialized approaches such as optical super-resolution.

Test specimens were created by culturing human cells with quantum dots. Optical sectioning was performed, which importantly does not harm the specimens, and simply takes advantage of the narrow depth of field inherent in the optical system. Phase contrast and confocal methods were not applied, so that simplicity and low cost could be demonstrated. Three-dimensional reconstruction was performed on a desktop Pentium 4 2.8 GHz processor, 512 MB RAM and 32 MB video RAM system.

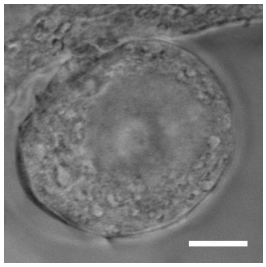

Figure 1

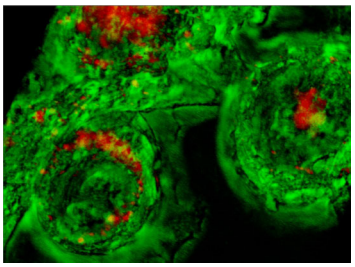

Figure 2

Figure 1 is an example of a single optical section of a cell, taken with 412 nm transmitted light from a 100 W tungsten-halogen lamp using a 30 second exposure time. The white scale bar is 5 µm long. This demonstrates that dominantly blue or intense laser sources were not necessary to achieve resolution slightly better than 300 nm. Nanoparticle positions were localized relative to cell features by combining the transmitted light images with epi-fluorescence images. After reconstruction and surface-modeling, images such as those shown in Figure 2 were used to distinguish and locate nanoparticles relative to cell features. ***Figure 2 is a virtual cross section into 3-D reconstructed cells (green) showing internal arrangement of nanoparticles (red). To aid visibility, the z-axis has been exaggerated by a factor of about 2.5. The field of view is nominally 40 µm wide.*** The ability to reconstruct multiple cells is of advantage to skin tissue studies.

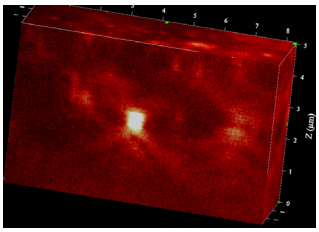

Figure 3

Figure 3 shows a virtual cross-section through a three-dimensionally reconstructed image of an area inside of a single cell, showing an example of the dimensions of the feature produced by quantum dot fluorescence. The slice shown in the figure is 5 µm high, nominally 8 µm wide and 2 µm thick. Each voxel is 47 nm x 47 nm x 50 nm. A voxel is defined as ***volume pixel***, the smallest distinguishable box-shaped part of a three-dimensional image). Voxel interpolation may be able to achieve a precision in quantum dot location close to the

size of the quantum dot diameter, notably well below the optical resolution limit. The value of such precise quantum location by optical methods relative to the diffraction-limited location of cell features remains to be seen, since to utilize this information with regard to subcellular components, combination with TEM methods may be required.

These results demonstrate that a simple optical microscope using inexpensive white-light sources, a liquid crystal tunable filter, and 3-D reconstruction with a modern desktop system can be used to image the location of quantum dot nanoparticles outside and inside cells with a resolution of slightly better than 300 nm. However, also suggested is that voxel interpolation may improve quantum dot location precision to less than 50 nm, below the diffraction limit. This presents a simplified but effective approach applicable to imaging nanoparticle and cell systems, and all equipment should be readily available to researchers who cannot afford laser-based, confocal, or electron microscopy systems. The more expensive and destructive methods of electron microscopy could potentially be used at a later point in product development and testing, thus providing research flexibility and saving the company time and money.

Future Plans: The next testing phase will include skin cells on scaffold systems, biofilms, and may include live systems, all of which are expected to offer special imaging challenges. Studies to explore support for focused ion beam (FIB) specimen preparation and TEM are planned.

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