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Tracking Nanoparticles the Easy Way

David L. Shenkenberg

Visualizing single nanoparticles and following them in three dimensions is useful for scientific applications ranging from examining biological cells to studying mixing in microfluidics. However, tracking nanoparticles in 3-D requires imaging outside of the focal plane, resulting in a loss of focus. Although setups with multiple cameras and beamsplitter mirror arrays can produce sharp 3-D images, the expense and complexity of such setups have prevented their widespread acceptance.

Now researchers at the University of Illinois at Urbana-Champaign have tracked nanoparticles in 3-D with accuracy as keen as several nanometers and temporal resolution down to several milliseconds, primarily using only one beamsplitter, a microscope and a single CCD camera.

In multicamera setups, the cameras can add up to $100,000 if each one costs about $20,000, said postdoctoral fellow Hamza Balci. He added that these setups are inconvenient because the cameras must be synchronized and identical because different specifications make analysis difficult.

To achieve high accuracy and temporal resolution, the researchers combined focused and defocused imaging. Basic in-focus microscopy works well in 2-D but cannot provide positional information in the Z-direction. On the other hand, defocused imaging exploits the loss of focus that occurs in the third dimension, which results in ring-shaped aberrations. The radius of these rings is directly related to the depth of a nanoparticle. Although defocused imaging can accurately determine the position of a nanoparticle in the Z-direction, it fares less well with X-Y-coordinates. Thus, focused and defocused imaging are complementary.

To image the nanoparticles, the scientists used an Olympus microscope with an Andor Technology CCD camera and, to the side port of the microscope, they attached a modified cassette from the Dual-View adapter made by Optical Insights LLC of Tucson, Ariz. The cassette enabled the researchers to simultaneously perform focused and defocused imaging. Basically, it is a beamsplitter and a holder. The beamsplitter sent the focused image to one half of the camera and the defocused image to the other half (Figure 1). The cassette created distance between the camera and the microscope, putting the image out of focus. To focus part of the

image after it travels through the beamsplitter, the researchers inserted a focusing lens into the cassette.

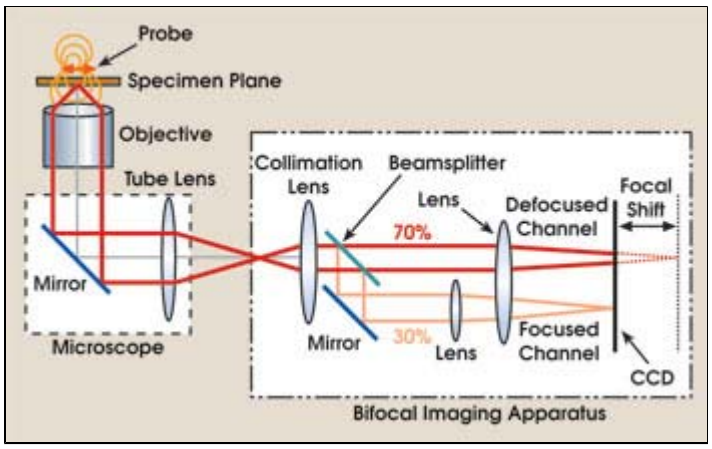


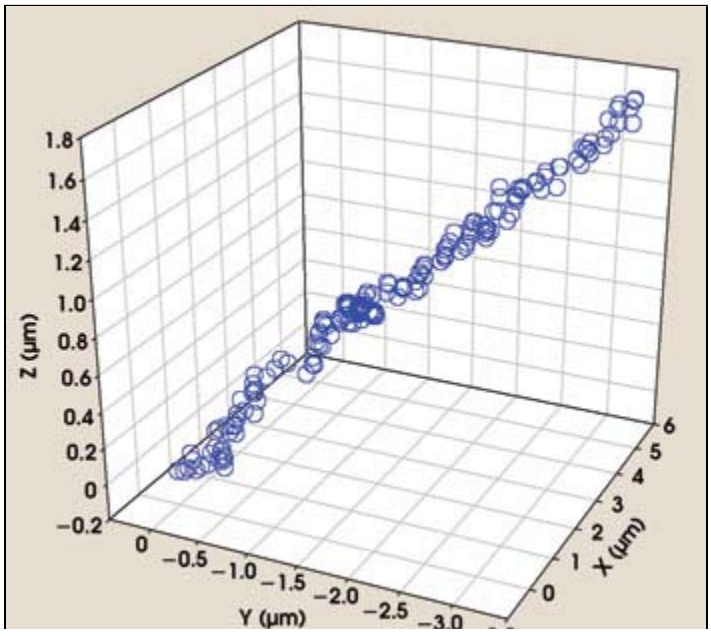
Figure 1. *A beamsplitter and three lenses enabled researchers to track nanoparticles in 3-D with high accuracy and temporal resolution using only a single microscope and a CCD camera. Image reprinted with permission of Nano Letters.*

They replaced the beamsplitter that came with the cassette with another that divided the beam intensity 30:70 to devote more photons to the defocused image, which is more difficult to interpret, according to Balci. In defocused images, several rings can appear, complicating the analysis, whereas focused images only require using a Gaussian point-spread function on the data.

Balci noted that, although the microimager requires an initial investment, the cassettes are relatively cheap. He also said that the cassettes are convenient because they are easy to modify.

In their experiment, the scientists tracked polystyrene beads moved in 3-D with a piezoelectric stage from Mad City Labs Inc. of Madison, Wis. (Figure 2). They also imaged polystyrene beads traveling along an inclined plane created from biological motor proteins on steps made from a polymer. They ultimately used the technique to image living cells, and the technique worked well for this application.

Figure 2. *This representative 3-D trace of a polystyrene bead displaced with a piezoelectric stage was made with the imaging setup.*



They also imaged quantum dots. However, they found that they needed to replace the focusing lens with a cylindrical lens because quantum dots are elongated and have a well-defined dipole moment. Thus, quantum dot emission has different shapes, depending on the orientation of the quantum dots with respect to the

polarization of the excitation laser.

The investigators plan to modify their imaging setup so that it provides information about the orientation of the quantum dots.

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