3D Particle Tracking in Bifocal Imaging Mode

3-Dimensional tracking of particles poses a challenge in terms of accurately measuring the Z-axis position. Typically it requires the acquisition of multiple images in different focal planes (using expensive scanning devices) as a function of time. The Z-stacks thus obtained comprise the 4D dataset (x,y,z,t) that the tracking information is usually extracted from. Acquisition of Z-stacks is time-consuming and reduces the time-resolution of the data.

The technique using the Dual View (http://www.photometrics.com/products/multichannel) introduced by Paul Selvin's group at the University of Illinois at Urbana Champaign simplifies the measurement considerably by employing simultaneous bifocal imaging with no moving parts.

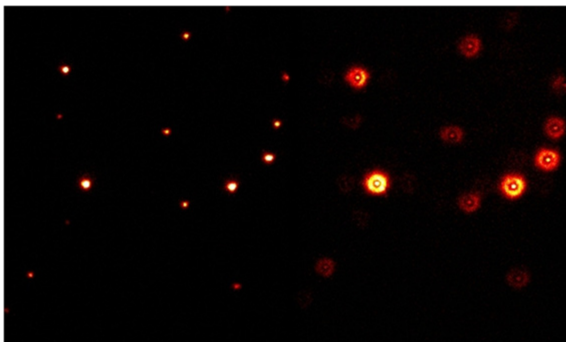

Figure 1. Images of 20 nm Nile red fluorescent beads using the Dual View apparatus. Total magnification is 90X, 30% of the emission is sent to the focused channel (left) and 70% of the emission is sent to the defocused channel. Focal shift is ~600 nm, achieved by inserting a spherical singlet lens with 250mm focal length (1″ diameter) in the right-hand side channel.

The basis of the Z coordinate measurement is the fact that a diffraction-limited point source will lead to the appearance of diffraction rings in the out-of-focus plane. The diameter of these rings is proportional to the Z distance from the focus within a range of several microns. The calibration is done using the first appearing ring. More rings will appear if the sample is moved further. The X and Y coordinates are determined from the focused image (left).

Calibration

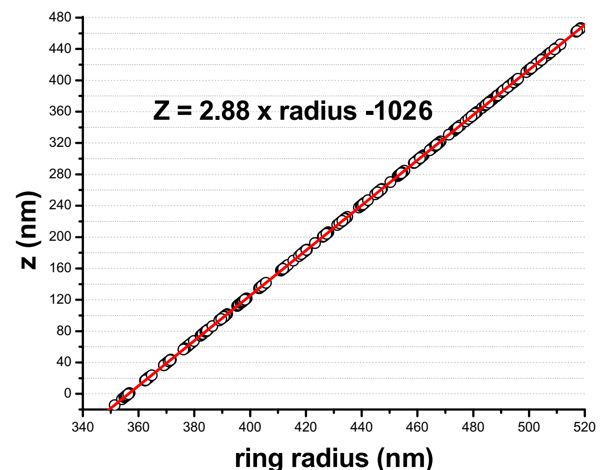

Figure 2. Calibration data for a 20 nm fluorescent bead displaced by a piezo-electric stage along the Z-axis. The displacement steps are plotted along with the corresponding ring radius. The conversion factor is extracted from the slope and intercept of the resulting linear fit.

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3D Trajectory

An example is an application involving melanosomes. They are nonfluorescent organelles, which carry the pigment melanin in melanophore cells. Figure 3 shows a melanophore after treatment with melanocyte-stimulating hormone (MSH). The hormone disperses the melanosomes to the cell periphery, thus it enables the tracking of individual organelles. The tracks themselves offer an insight into the features and mechanisms of intracellular transport.

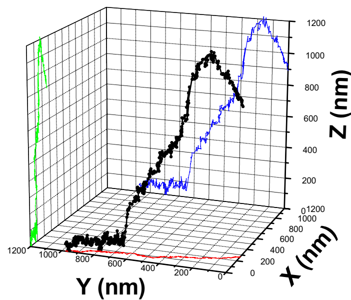

Figure 3. 3D motility trajectory of a melanosome organelle moving across the cytoskeletal tracks inside a cell (black). It would normally appear as its projection on the XY plane (red). Melanosomes are nonfluorescent, demonstrating that the technique can equally well be applied to fluorescent and nonfluorescent particles.

Optical path through the Dual View

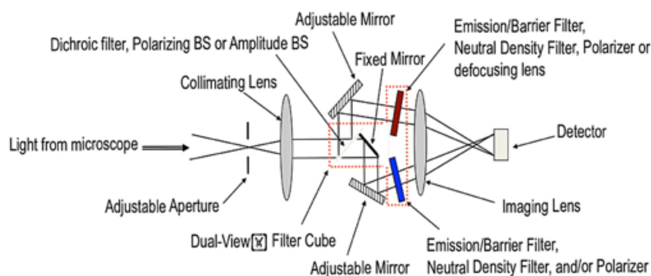

Figure 4. Schematic drawing of the Dual View. Of the available options the Dual View casette was equipped with an amplitude beamsplitter to separate the light from the microscope into two beams in a 30:70 intensity ratio. A defocusing lens was added in one of he two beampaths (red element within the casette outline). The choice of lens focal length depends on the microscope objective magnification and Z-axis range studied.

References

1. Erdal Toprak, Hamza Balci, Benjamin H. Blehm, Paul R. Selvin. Three-Dimensional Particle Tracking via Bifocal Imaging. Nano Lett 7 (7), 2043–2045, 2007.
2. M. Speidel, A. Jonas, and E. L. Florin, Opt Lett 28 (2), 69 (2003).

RESOURCES

To learn more about the Photometrics DV2, visit **www.photometrics.com/products/multichannel**.

To learn more about high-performance CCD cameras, visit **www.photometrics.com** and **www.qimaging.com**.

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