Supplementary Figures

3D Particle Tracking via Bifocal Imaging
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Figure S1

The graph shows a linear relationship between Z Position (nm) and Radius (pixel), described by the equation:

\[ Z = -981.3 + 249.0 \times R \]

The data points are represented by circles, and the best-fit line is shown in red.
Fig. S2

(a) \[ y = y_0 + \frac{A}{(w \sqrt{\pi/2})} \exp\left(-\frac{2((x-x_c)/w)^2}{w} \right) \]

- \( y_0 = 1.2 \pm 1.9 \)
- \( x_c = 0.0 \pm 0.0 \)
- \( w = 2.7 \pm 0.1 \)
- \( A = 340.8 \pm 16.1 \)

(b) \[ y = y_0 + \frac{A}{(w \sqrt{\pi/2})} \exp\left(-\frac{2((x-x_c)/w)^2}{w} \right) \]

- \( y_0 = 4.6 \pm 2.8 \)
- \( x_c = -0.1 \pm 0.1 \)
- \( w = 3.7 \pm 0.2 \)
- \( A = 289.9 \pm 27.8 \)
Fig. S4

(a) Polymer step

(b) Axoneme

(c) Kinesin coated beads

(d) Path

(e) Graph showing position vs. time

Position (µm) vs. Time (sec)

X, Y, and Z components are plotted.
Average = 40.3 ± 5.6 nm
Fig. S6

\( x_c = -0.20 \pm 0.01 \)
\( \sigma = 0.05 \pm 0.01 \)
SCHEMATICS OF SIMULTANEOUS BIFOCAL IMAGING APPARATUS

Images are projected side-by-side on CCD chip.

AR coated spherical singlet biconvex lens (f=225mm).

30:70 mirror (optical insights ms-30/70-1826)

Collimating Lens

Incoming Light