

# 3D POSITIONAL ANALYSIS OF MOVING NANOSCALE FLUORESCENT OBJECTS NEAR REFLECTING SURFACES

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Several strategies have been described recently to track single fluorescent particles in the x-y plane with nm-accuracy. For precise measurements in the z-direction, sectioning or defocussing techniques are being used. Although nm-resolution can be achieved by these methods, their performance is limited in speed or sensitivity. Alternatively, high-resolution z-measurements can be based on fluorescence interference contrast (FLIC) where interferences of excitation and emission light above a reflecting surface lead to a height dependent modulation of the fluorescence signal (see Figure 1). FLIC microscopy has so far mainly been used for height measurements of membranes above surfaces [1].

Here, we report on the application of widefield FLIC microscopy to the 3D positional analysis of moving nanoscale objects. Using a self-calibrating method, nm-height measurements can be performed on unstructured reflecting surfaces using any kind of epi-fluorescent microscope. In particular, we determine the height at which kinesin-driven microtubules glide over a surface and we elucidate the geometry of motile microtubules crossing each other. Combined with high resolution x-y tracking the 3D positions of many fluorescent nanoobjects can be determined simultaneously with nm-precision. Such data is crucial for the nanotechnological design of kinesin-driven transport systems, as well as for a fundamental understanding of how a motor protein interacts with its filamentous track.

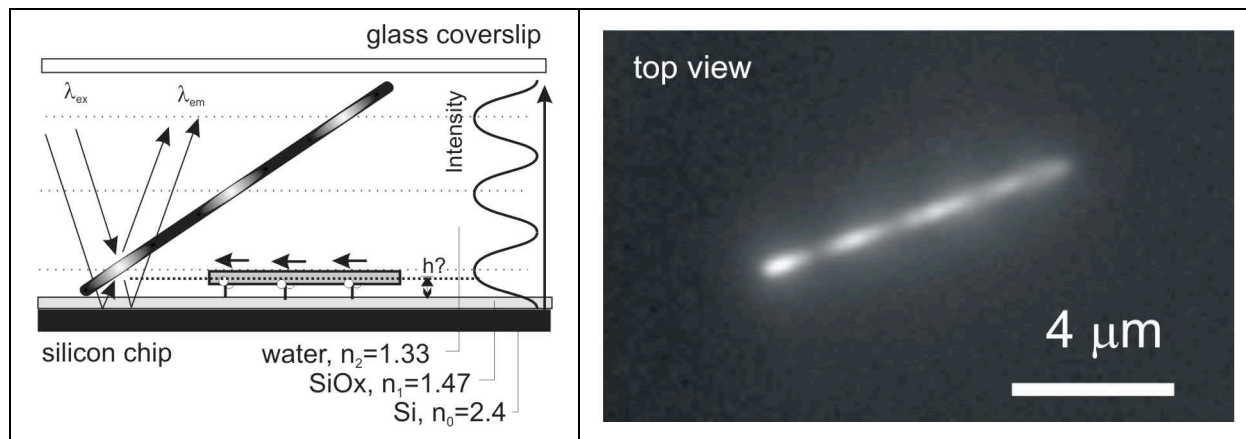


Fig. 1 Principle of quantitative FLIC microscopy on microtubules. The height of a moving microtubule can be obtained by mapping its intensity onto that of a fixed, tilted microtubule (left). Fluorescent image of a tilted microtubule as captured on the CCD camera chip (right).

## References:

- [1] Braun, D., and Fromherz, P. (1998). Fluorescence interferometry of neuronal cell adhesion on microstructured silicon. *Physical Review Letters* 81, 5241-5244.