Universität Bielefeld

Video-based Axial Force Analysis for 3D Quantitative Optical Tweezers

Sebastian Knust[1*], Andy Sischka[1], Andre Spiering[1], Christoph Pelargus[1], Dario Anselmetti[1]

[1]Experimental Biophysics and Applied Nanoscience, Bielefeld University, 33615 Bielefeld, Germany

* sknust@physik.uni-bielefeld.de

Experimental Biophysics Group
BIELEFELD UNIVERSITY
CENTER FOR BIOTECHNOLOGY (CEBITEC)
BIELEFELD INSTITUTE FOR BIOPHYSICS & NANOSCIENCE (BINAS)

Abstract

We developed and included video-based axial force analysis into our previously described optical tweezers setup [1]. By measuring the radius of a trapped microbead we achieve an overall force resolution along the z-axis in the range of 0.2pN with a bandwidth of 120Hz, only limited by our CCD camera. With this video-based method we overcome the remaining weak interference effects in backscattered light based force analysis when operating a microsphere in the vicinity of an interface.

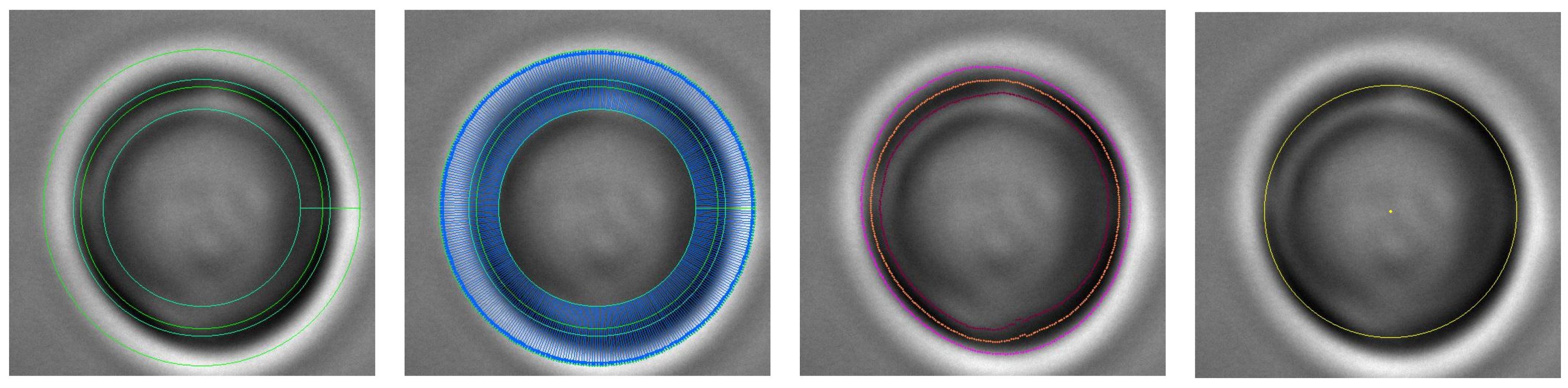
We tested our setup by investigating the controlled threading and translocation of individual lambda-DNA molecules with and without attached DNA-binding ligands through solid-state nanopores and comparing these results with previous measurements realized with photodiode intensity detection [2, 3].

Theory

Optical tweezers generate a three dimensional harmonic potential. Applying an axial force at an optically trapped microbead moves the bead out of the trap and thereby defocuses it. This can be used to measure the applied force, either by analyzing the intensity of the transmitted or reflected trapping light, or by monitoring the apparent size of the bead.

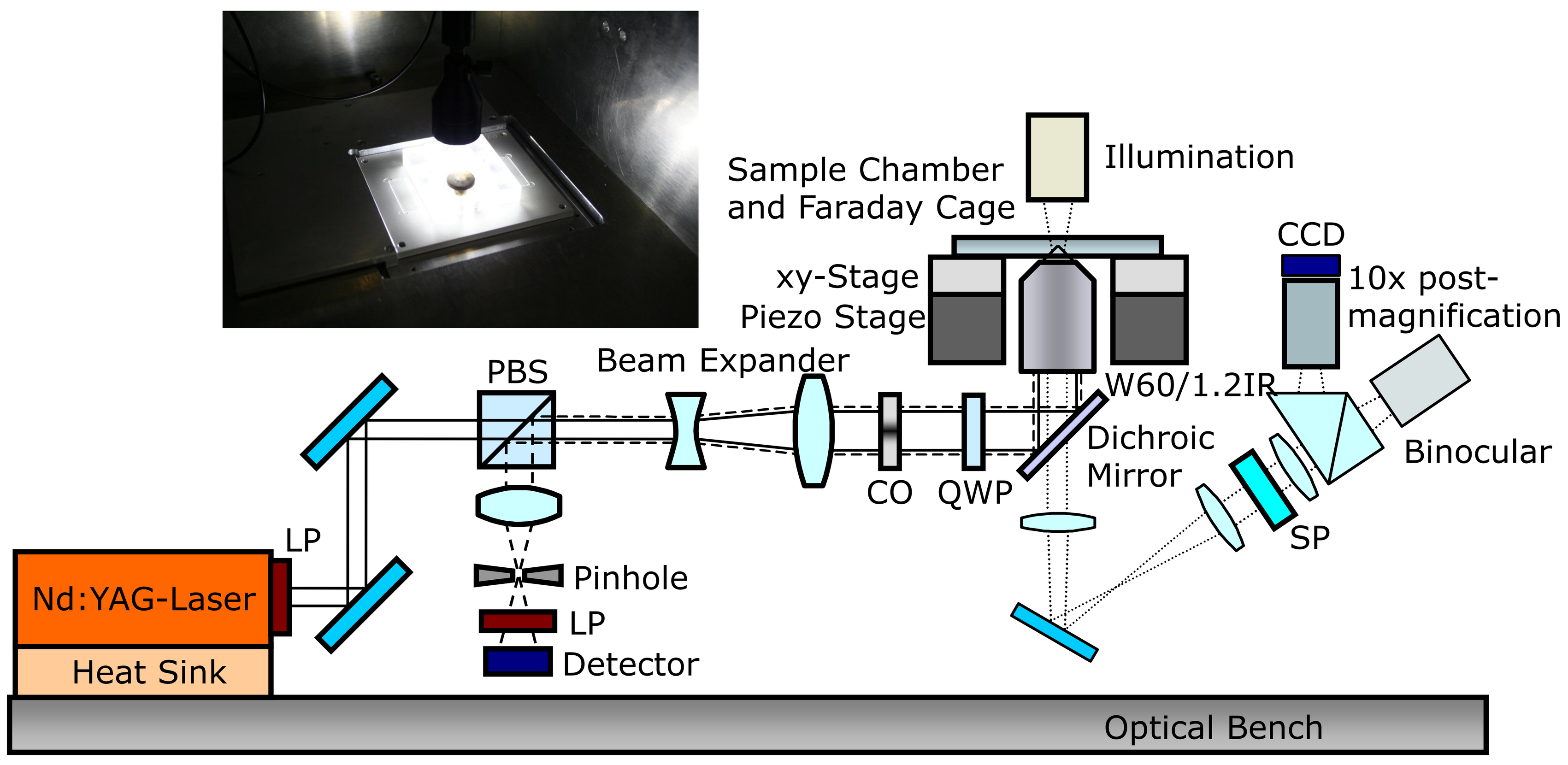
We measure the size of the microbead with a commercial CCD camera using a total magnification factor of approximately 600 and fiber-optical illumination. The size is determined by edge detection: The intensity profile along multiple spokes in a circular ROI is interpolated and the steepest rising and falling slopes are averaged.

Size Detection



Separate circular ROIs for rising (green) and falling (teal) edges | Detect edges along 360 spokes in ROI | Average (yellow) rising (magenta) and falling (violet) edges | Fit circle through averaged points and determine radius

Experimental Setup

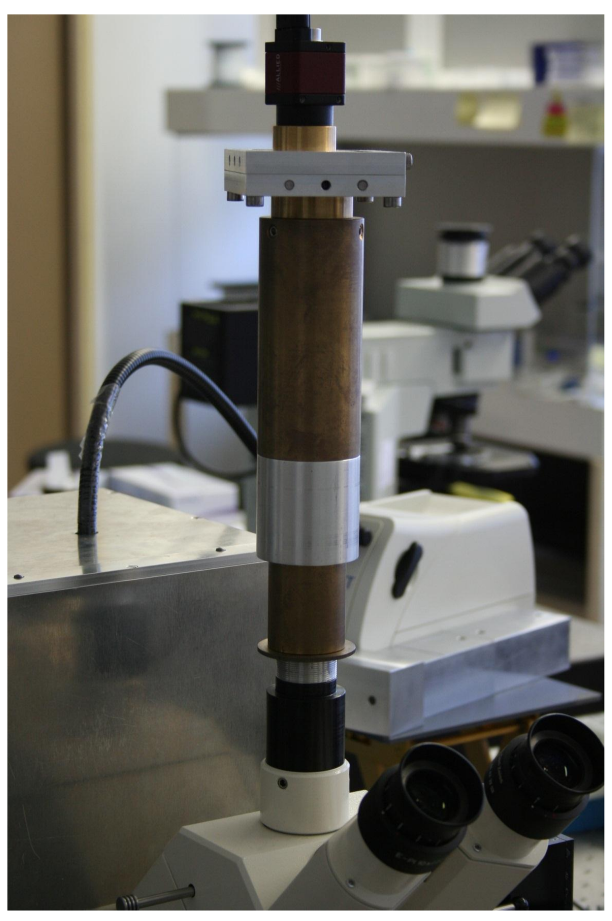


For illumination, we use a Schott KL2500 LED cold light source with a maximum output of 1000lm. Currently, due to contacting we cannot place the fiber directly onto the sample chamber. Therefore we focus the light with an additional lens.

For image acquisition, we use a custom built post-magnification with a magnifying factor of 10 and an AVT Guppy Pro F-031 monochrome firewire CCD camera with 14bit image acquisition at 123fps.

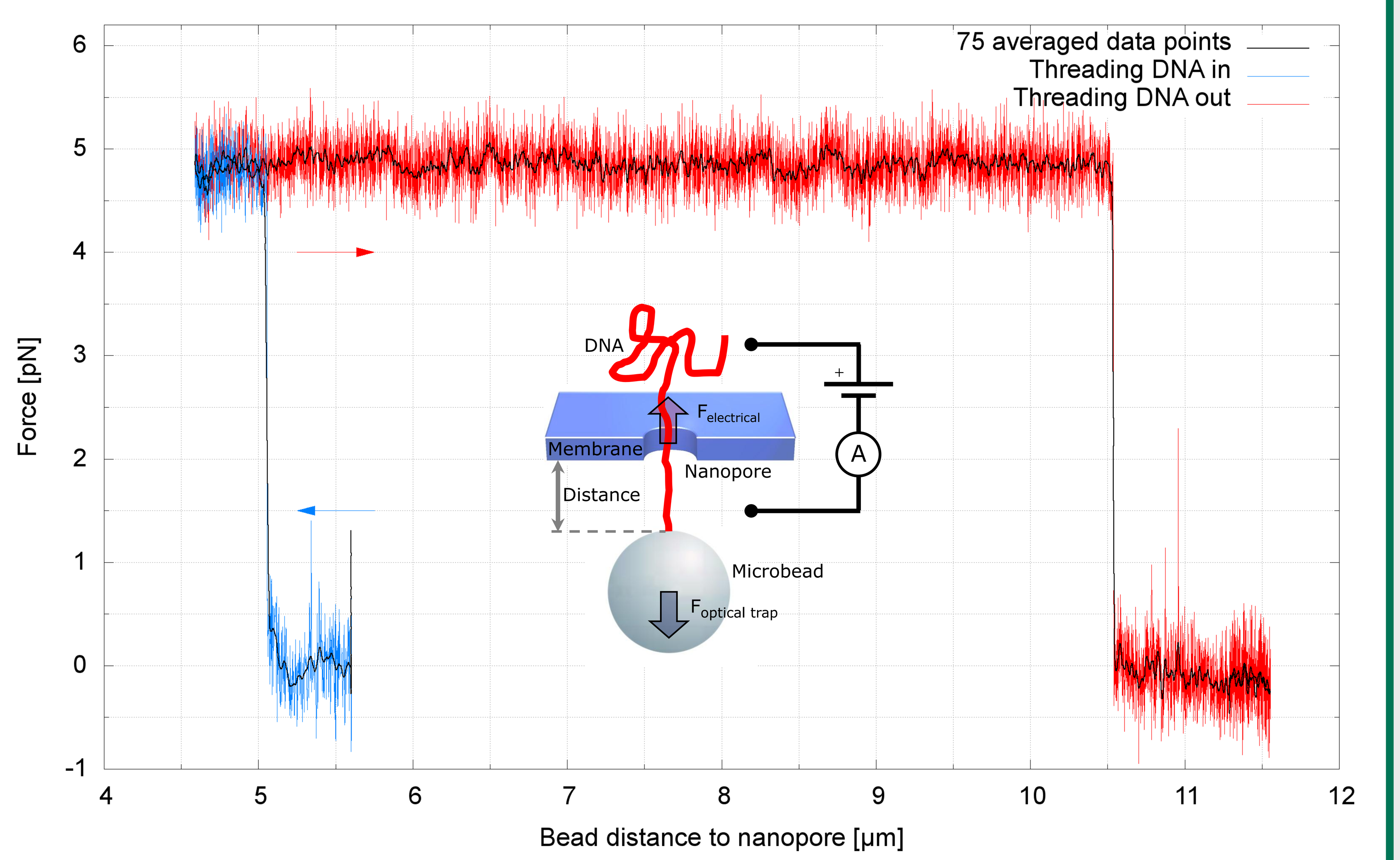
With the pixel size of 5.6µm, we get a conversion factor of approximately 9nm/px. The bead has an apparent radius in the range of 170px.

A 1pN force corresponds to a bead radius change of approximately 0.8px at 250mW laser power.

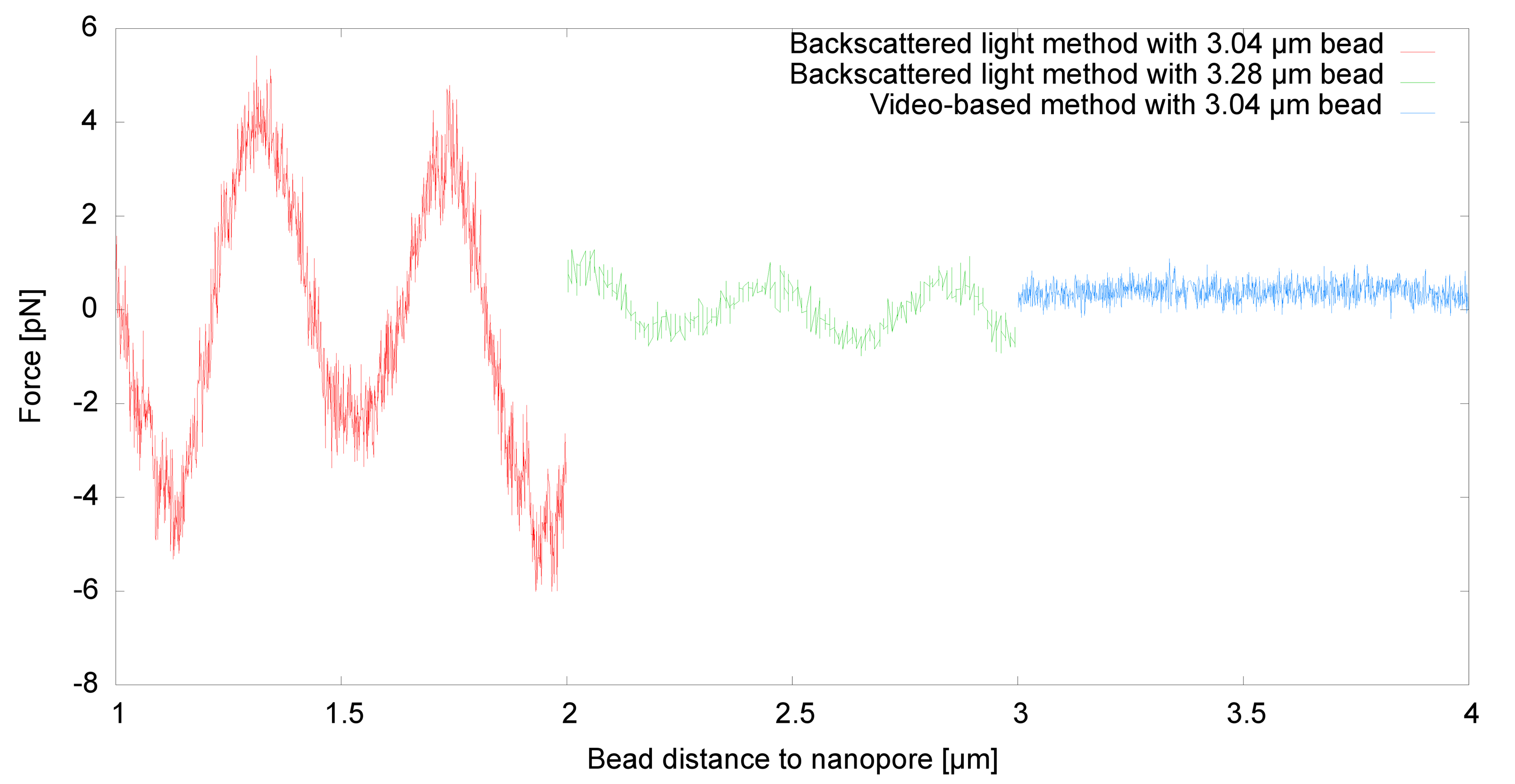


Results

3.04 µm bead in vicinity of Si_3N_4 nanopore with applied voltage of 50 mV, laser power 250 mW



Comparison of interference effects



Conclusion

Our video-based force analysis system is capable of performing axial force measurements up to approximately 10pN with a force resolution of up to 0.2pN. The maximum force range can be extended to approximately 70pN with a remaining resolution of 1.5pN.

Currently, the camera is the limiting factor both in bandwidth due to the limited framerate and in resolution due to relatively high noise. However, better cameras usually have larger pixels, which might impede the force resolution due to smaller radii.

All calculations are carried out by a hexacore CPU near the limit of performance.

References

[1] A. Sischka et. al., Rev. Sci. Instrum. **79**, 063702 (2008)
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[3] A. Spiering et. al., Nano Lett. **11**, 2978 (2011)

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