

In vivo single molecule tracking with light sheet-based microscopy

Jörg G. Ritter, Roman Veith, Jan Peter Siebrasse and Ulrich Kubitschek
Institute of Physical and Theoretical Chemistry,
Wegelerstr. 12, 53115 Bonn, Germany
E-Mail: ritter@pc.uni-bonn.de

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Autofluorescence, rapid photobleaching and high particle concentrations present restrictions to single molecule observation in biological systems by epi-illumination. Light sheet-based microscopy overcomes these limitations [1]. By combining the speed of parallel image acquisition and the optical sectioning produced by light sheet illumination, we created a powerful tool to study single molecule dynamics on a millisecond timescale [2].

We illuminated the sample perpendicular to the detection axis with a thin light sheet (FWHM 2-3 μm). In this manner a simple optical sectioning microscope is created, because only the focal plane of the detection optics is illuminated and no out-of-focus fluorescence is generated. The background fluorescence is strongly reduced and the signal-to-noise-ratio (SNR) greatly improved.

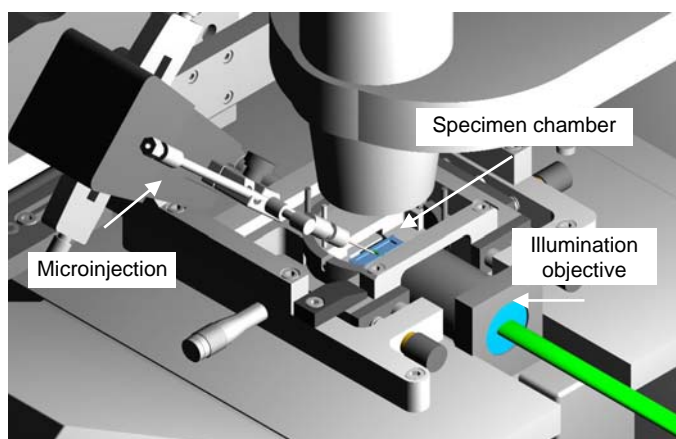


Figure 1: Setup with miniaturized glass specimen chamber

We constructed a miniaturized glass specimen chamber, which can be illuminated from the side in a very flexible manner and directly be mounted on a commercial inverse microscope. The specimen is easily accessible from above for (Figure 1) micromanipulation and can be observed via the 0.17mm thin glass bottom of the chamber using high NA objective lenses.

It was straightforward to observe trajectories of single protein molecules in aqueous solution with a $D = 90\mu\text{m}^2/\text{s}$, and also in the cellular interior, with unprecedented imaging speed and signal-to-noise ratio. The technique is ideally suited for single molecule imaging in living cells. By analysis of the diffusion behavior of single fluorescent dextran molecules we determined the viscosity of living *C. tentans* salivary gland cell nuclei. Furthermore, we were capable to observe and analyze the movement of single mRNA particles, labeled with microinjected hrp36 proteins, in this system. Similarly, molecular dynamics of fast moving single Ovalbumin molecules (43Kd) in adherent, living Hela cells could be observed with greatly improved contrast.

With this new experimental setup we use the ideal imaging scheme for single molecule visualization and push the limit of sensitivity far beyond the potential of conventional epi-illumination. Further improvements of the setup aim at three dimensional real-time single molecule tracking.

[1] Huisken, J. et al. *Science* **305**(5686): 1007-9 (2004).

[2] Ritter, J. G. et al. *Opt Express* **16**(10): 7142-52 (2008).