Key Words: Fluorescence microscopy, single molecule microscopy, optical sectioning, nuclear pore complex, nuclear transport, transport receptors, dwell times.

In eukaryotic cells all transport of proteins and RNA between cytosol and nucleus is mediated by the nuclear pore complexes (NPCs), which are embedded within the nuclear envelope. So far most studies addressing the dynamical behavior of different transport receptors at the NPC employed classical epi-illumination fluorescence microscopy [1, 2]. Here, a new microscopic approach is utilized, which is based on highly inclined and laminated optical (HILO) sheet microscopy [3]. We make use of a strongly refracted beam, which is obtained by hitting the coverslip/sample interface under an angle just slightly smaller than the critical angle of total reflection. Using this technique an optical sectioning effect similar as in light sheet microscopy is achieved [4, 5]. Thereby only the NPCs at the bottom of cell nuclei are illuminated, and single NPCs can be identified in a straightforward way because the out-of-focus fluorescence is strongly reduced and the signal-to-noise ratio greatly improved. We demonstrate that sample illumination is restricted to a region close to the cover slip surface, and quantify in detail the axial extension of the illumination volume.

In this study we focus on the dwell times of different transport receptors at the NPC in the absence or presence of their respective cargoes. In particular, we analyzed the binding site distribution of specific NPC transport receptors and of inert molecules, which can pass the nuclear pore complexes by passive diffusion, in relation the center of single NPCs.


