

# SINGLE MOLECULE MICROSCOPY OF NUCLEO-CYTOPLASMIC TRANSPORT

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In eukaryotic cells all transport of proteins and RNA between nucleus and cytoplasm is mediated through the nuclear pore complexes (NPC), which are embedded in the nuclear envelope. In previous studies using classical epi-illumination fluorescence microscopy we determined the dwell times of different transport receptors at the NPC [1, 2].

In this study we focus on the dwell times of the NTF2 transport receptor at the NPC under different physiological conditions; i.e. absence or presence of RanGDP, GTP and an ATP regenerating system. Additionally we perform measurements with a NTF2-mutant, which cannot interact with FG-repeats.

In our experiments we use an illumination method similar to HILO microscopy [3]. We make use of a strongly refracted beam to exclusively illuminate the bottom of the cell nucleus. We obtain this beam by hitting the coverslip/sample interface under an angle just slightly smaller than the critical angle of total reflection. Using this technique we achieve an optical sectioning effect and therefore only the NPCs at the bottom of the nucleus are illuminated and thus only transport receptors binding to them are detected. By this means the out-of-focus fluorescence is strongly reduced and the signal-to-noise ratio improved [Fig. 1]. Additionally, looking exclusively at the bottom plane of the nucleus, it is much easier to identify single NPCs.

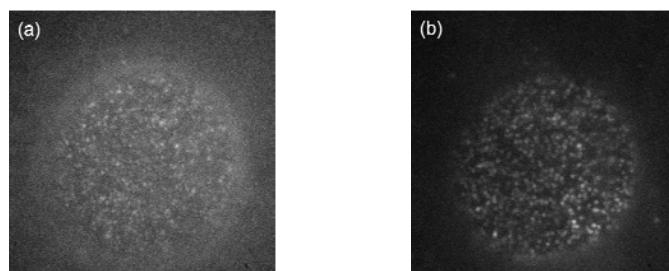


Figure 1: (a) EPI-Illumination, (b) HILO-Illumination

[1] U. Kubitscheck, D. Grünwald, A. Hoekstra, D. Rohleder, T. Kues, J.P. Siebrasse, and R. Peters, „Nuclear transport of single molecules: dwell times at the nuclear pore complex”, *The Journal of Cell Biology*, **Vol. 168**, No. 2, January 17, 233–243 (2005).

[2] T. Dange, D. Grünwald, A. Grünwald, R. Peters, and U. Kubitscheck, “Autonomy and robustness of translocation through the nuclear pore complex: a single-molecule study”, *The Journal of Cell Biology*, **Vol. 183**, No. 1, 77–86 (2008).

[3] M. Tokunaga, N. Imamoto, and K. Sakate-Sogawa, “Highly inclined thin illumination enables clear single molecule imaging in cells”, *Nature Methods*, **Vol. 5**, 159 – 161 (2008).