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Single molecule imaging and quantification in the cell nucleus by reflected light sheet microscopy

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Single fluorescent molecule imaging in the mammalian nucleus is challenged by out-of-focus fluorescent background in common microscopy schemes. We report a novel illumination method, reflected light sheet microscopy (RLSM), that enables selective plane illumination throughout the nucleus of living mammalian cells [1]. Generation of a thin light sheet parallel to the imaging plane and close to the sample surface is achieved by reflecting an elliptical laser beam incident from the top by 90° with a small mirror (Figure 1). The thin light sheet allows for an increased signal-to-background ratio superior to previous illumination schemes and enables imaging of single fluorescent proteins with up to 100 Hz time resolution. With the new microscope we measured the DNA-bound fractions and residence times of the transcription factor glucocorticoid receptor (GR) and distinguished different modes of binding to DNA, including indirect binding via protein-protein interactions. Analogous experiments on estrogen receptor (ER) yielded similar results. Moreover, we demonstrated two-color single molecule imaging by observing the spatio-temporal co-localization of interacting protein pairs [1]. In a second set of experiments, we combined RLSM with superresolving stochastic optical reconstruction microscopy (STORM) imaging and molecular counting, and quantitatively mapped the positions and the abundance of RNA polymerase II throughout the nucleus. We found a homogenous distribution, inconsistent with the model of transcription factories.

1. J.C.M. Gebhardt*, D.M. Suter*, R. Roy, Z.W. Zhao, A.R. Chapman, S. Basu, T. Maniatis, and X.S. Xie, Nature Methods 10 (2013), p421-426

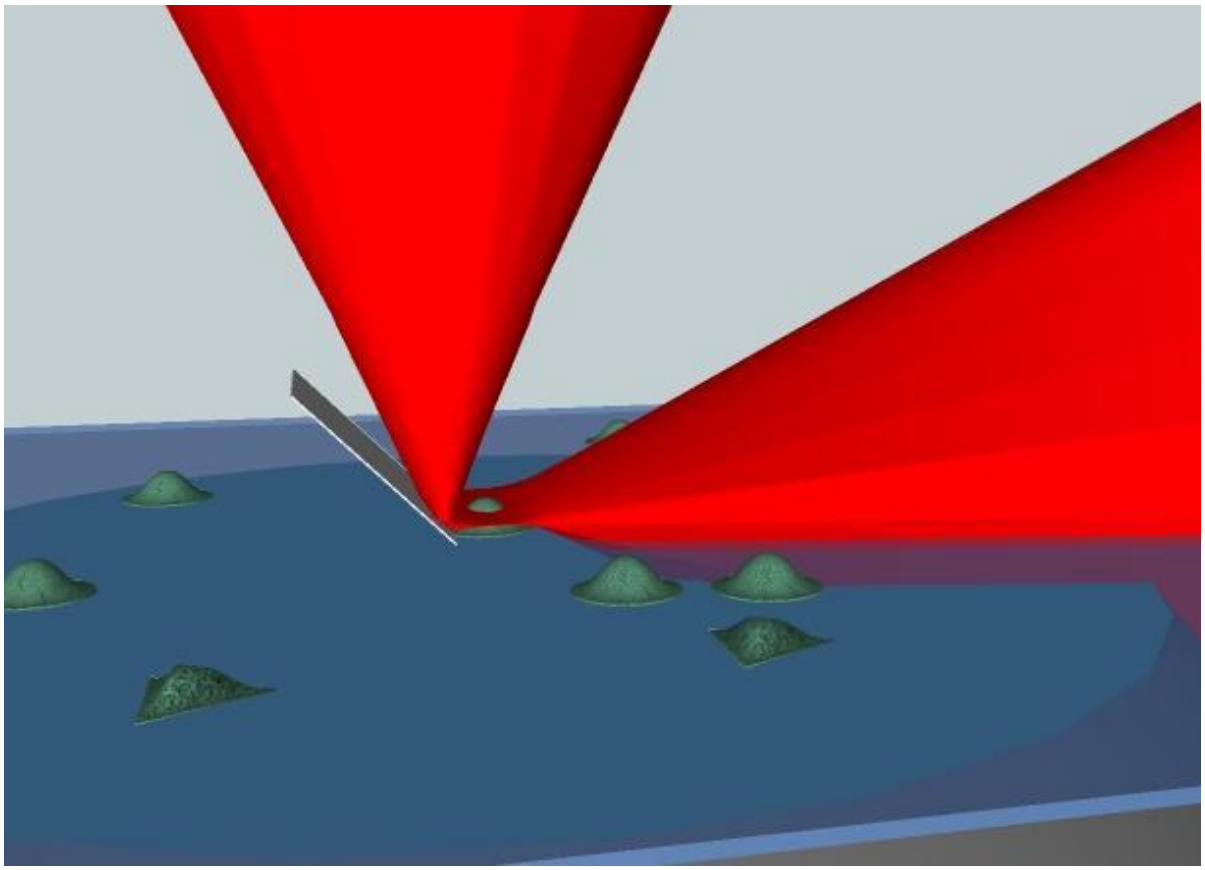

Figure. Scheme of the principle of reflected light sheet microscopy