INTRINSIC REFRACTIVE INDEX MATCHED 3D DSTORM WITH TWO OBJECTIVES

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In our custom-built microscope, we employ astigmatic and interferometric 3D dSTORM in conjunction with glycerol based refractive index matching [1]. Glycerol-immersion objectives, fused silica cover glasses and glycerol-based immersion media as well as imaging buffers prevent refractive index mismatches, which greatly reduces aberrations, especially deep in the sample. We have shown that the high glycerol content in our imaging buffer does not negatively affect the switching behaviour of our fluorescent dyes.

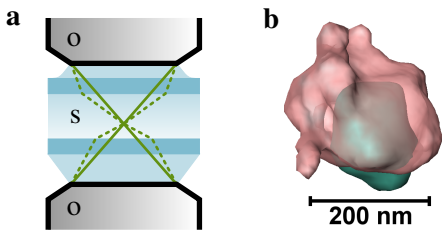

Figure 1: **a:** Beam path with (dashed) and without (solid) refractive index mismatch. o: objective, s: sample. **b:** Nuclear pore complex. Outer structure (red): Nup358, inner structure (cyan): mAb414.

Additionally, our microscope detects the fluorescent light via two objectives (see Fig. 1a), thus doubling the number of photons and improving the localisation precision [2, 3]. For such a configuration, refractive index matching is especially beneficial as the second objective has to image the sample from the other side, collecting the light through a more than 15 μm deep layer of imaging buffer.

This setup permits 3D imaging with excellent localisation precision deep inside samples. Recently, we have resolved HeLa cell nuclear pore complexes across the whole nucleus in two colours (see Fig. 1b).

References

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