

REALM: AO-BASED LOCALIZATION MICROSCOPY DEEP IN COMPLEX TISSUE

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1. ABSTRACT

While Single-Molecule Localization Microscopy (SMLM) has provided many new insights into the organization of individual cultured cells, performing SMLM deep inside tissue (>10 μm) has remained challenging. Sample-induced aberrations and scattering reduce contrast and hamper detection and accurate localization. A way to overcome this is the use of image-based Adaptive Optics (AO). However, in SMLM the acquisitions are noisy and contain a strongly fluctuating amount of signal photons, rendering traditional approaches unusable.

Here we systematically compared the performance of three recently proposed SMLM-specific AO-methods [1-3] and found that these methods provide no or only limited correction. Careful analysis of the reasons that underlie this limited success enabled us to develop a new method, we termed REALM (Robust and Effective Adaptive Optics in Localization Microscopy).

We demonstrate the improvement of REALM by imaging stained COS-7 cells through 50 μm thick rat brain slices in and observe an up to 6-fold improvement in the number of localizations. Finally, we imaged the axon initial segment (AIS) in rat cortical brain slices. Using REALM, we performed multiplane 3D astigmatic SMLM imaging on βIV -spectrin stained brain sections up to a depth of 50 μm , which enabled us to resolve the periodic patterning of this scaffolding protein in 3D and revealed a periodicity of 203 ± 10 nm (mean \pm sd).

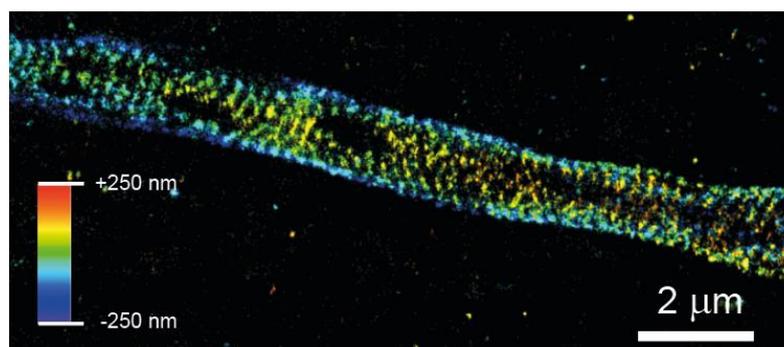


Figure 1. SMLM reconstruction of βIV spectrin in a rat brain slice at 50 μm depth.

[1] D. Burke, et al., "Adaptive optics correction of specimen-induced aberrations in single-molecule switching microscopy," *Optica* (2015).

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