Adaptive Holographic Region of Interest Illumination with Oblique Angles for use in Fluorescence Super-Resolution Microscopy

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Abstract

To improve the localization accuracy beyond the current limit and to reduce the overall photo-damage in Single Molecule Localization Microscopy [1], we introduce a holography based region-of interest (ROI) illumination with two different application modes basing on a recently shown approach [2]. Both modes are realized in the same setup but with different advantages and application scenarios. By illuminating a phase-only spatial light modulator (SLM) (Fig. 1a) twice, both modes allow to adaptively modify the size and the (excitation) angle of the ROI illumination. This results in a reduced out-of focus signal and less overall phototoxicity. The first mode (Fig. 1c) generates an (almost) speckle-free circular ROI with selectable size and illumination angle. It is realized by focussing the excitation beam with different spot sizes and lateral position in the back focal plane (BFP) of the objective. SLM1 and SLM2 are working therefore like an auto-collimator to change the diameter of the incoming beam (Fig. 1b). The second mode allows more flexibility with respect to the shape of the ROIs at the cost of more speckles in the illumination and longer calculation times. An Iterative Light Propagating Algorithm retrieves the phase distribution which the SLM displays to generate the desired user defined pattern (Fig. 1d, e). This mode reduces the overall phototoxicity in case of non-circular structures better than mode 1. By adding in both modes specific phase terms to the holograms, illumination angles up to 60° are realizable with respect to the optical axis, using high NA objectives.

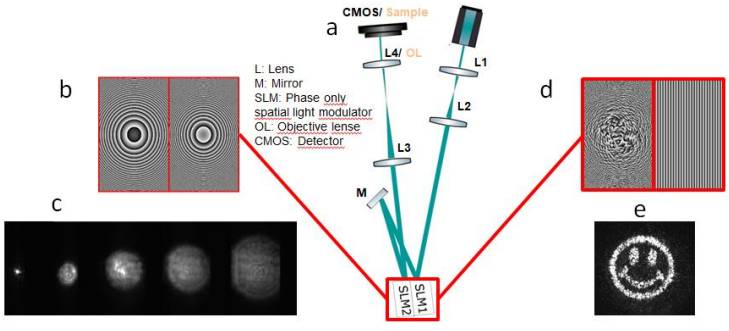

Figure 1: By illuminating a SLM twice (a) two different illumination modes can be realized. By generating specific focus, defocus and superimposed phase-ramp holograms (b) (near) speckle free, circular ROI illumination with selective size and oblique angles can be realized (c). By generating holograms (d) using an Iterative Light Propagation Algorithm user defined patterns (e) with oblique angels can be realized. Tilt angles up to 60° with respect to the optical axis (using high NA objectives) are possible.

References:
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