holoMINFLUX – Super-Resolution Imaging with Several Independent Zeros

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Abstract

Great improvements in Single Molecule Localization Microscopy (SMLM) were achieved with the development of MINFLUX [1] and SIMFLUX [2]. For localization, the response of the fluorophores is used after illuminated with a pattern containing one precisely controllable zero (MINFLUX) or several zeros which cannot controlled individually (SIMFLUX), resulting in higher acquisition speed but less localization accuracy. Here we present first experimental results of a fluorescence widefield technique combining advantages of both techniques. With holoMINFLUX, we show that we are able to produce several, independent zero intensities in a rather simple setup using a Phase-Only Spatial Light Modulator (SLM). At the assumed positions of the single fluorophores (Fig. 1A) we generate patterns (Fig. 1E) with sharply defined zeros. Therefore, the SLM generates on the corresponding positions (Fig. 1C) holograms with two or more parts with relative phase differences of π at the touching edges. The easiest example of a possible hologram is a "Phase-Box" containing only two halves (Fig. 1B). At the position of the zero intensity, the complex amplitudes of the incoming light manipulated on each half will interfere destructively. This results in a zero transition of the overall complex amplitude of the illumination light at the position of the zero. For subpixel accuracy we also need to manipulate the intensity of the light by e.g. integrating in one half of the "Phase Box" a grid with different modulation depths. In combination with a pupil plane filter in the beam path (Fig. 1D), different modulation depths of the grid correspond to different intensity manipulations. This results in different slight shifts of the zero transition. Using this approach it is possible to implement a parallel MINFLUX to localize the positions of the fluorophores with improved accuracy and reduced overall phototoxicity (Fig. 1F).

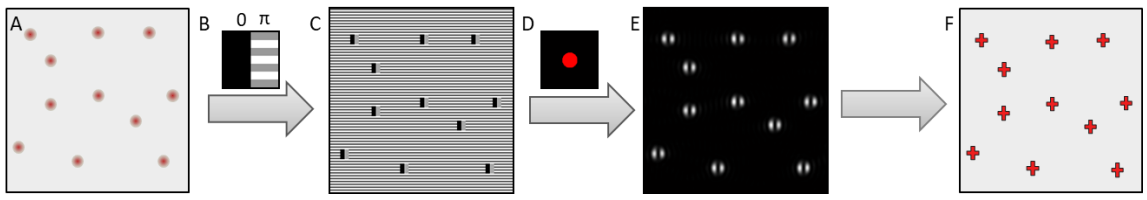

Figure 1: SMLM using holoMINFLUX. At the roughly determined positions of the fluorophores (A), "Phase-Boxes" (B) are placed at the corresponding position on a phase-only SLM (C). Due to the interaction of the incoming illumination light with the "Phase-Boxes" a pattern containing a zero at the fluorophore position is produced (E). To control the zero with subpixel accuracy, elements for intensity manipulation are included in the holograms in combination with a pupil plane filter in the beam path (D). Using the response of the fluorophore to slightly different positions of the adjacent zero, the positions of the fluorophores can be localized with an improved accuracy (F).

References:

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[2] Cnossen et al. "Localization microscopy at doubled precision with patterned illumination". Nat Methods 17, 59–63 (2020)