Multi-Focus microscopy with precise dual color imaging capabilities and enhanced sensitivity

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ABSTRACT:

The growing interest of the scientific community in single molecule imaging for biological studies raises many new challenges. One of the most pressing ones is the ability to image rapidly single molecules of multiple colors in the 3D extent of the biological specimens with the best signal to noise ratio. We have shown that multifocus microscopy (MFM) is capable of acquiring instantaneous 3D stacks without mechanical movement thanks to a custom made diffraction grating introduced on the emission path of a widefield microscope [1-4]. The grating splits the emission into nine images and adds different defocus degrees to each, allowing for the acquisition of instantaneous 3D stack. However, the grating performance is only guaranteed for a given wavelength.

Here we show a new MFM scheme for sensitive multicolor single molecule imaging. We designed new diffraction binary gratings with enhanced performances and exhibiting interesting dual color properties. A general approach for designing the grating will be discussed for various number of imaging planes. Reducing the number of imaging planes guarantees a larger number of photons collected per plane compared to the conventional 9 planes configuration. Examples will illustrate the advantage of this design for 3D imaging.

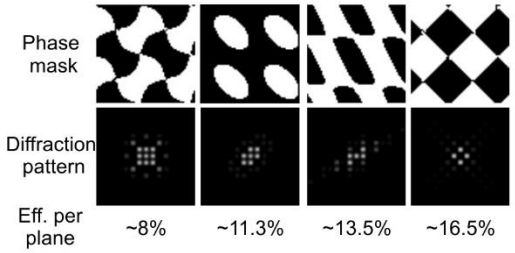

Figure 1: Different diffraction masks designs for multifocus microscopy allowing a flexible number of imaging planes and exhibiting multicolor properties.

REFERENCES:

1. Hajj, B., M. El Beheiry, and M. Dahan, *PSF engineering in multifocus microscopy for increased depth volumetric imaging.* Biomed Opt Express, 2016. **7**(3): p. 726-31.
2. Abrahamsson, S., et al., *Multifocus microscopy with precise color multi-phase diffractive optics applied in functional neuronal imaging.* Biomed Opt Express, 2016. **7**(3): p. 855-69.
3. Hajj, B., et al., *Whole-cell, multicolor superresolution imaging using volumetric multifocus microscopy.* Proc Natl Acad Sci U S A, 2014. **111**(49): p. 17480-5.
4. Abrahamsson, S., et al., *Fast multicolor 3D imaging using aberration-corrected multifocus microscopy.* Nat Methods, 2013. **10**(1): p. 60-3.