ADVANTAGES OF CIRCULAR POLARIZATION BASIS FOR PSF ENGINEERING

Isael HERRERA,[1] Luis A. ALEMAN-CASTANEDA[1], Sophie BRASSELET [1], Miguel A. ALONSO [*,1,2]

[1] *Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France;*
[2]*The Institute of Optics, University of Rochester, Rochester, NY, USA*
** miguel.alonso@fresnel.fr*

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In recent years there has been interest in improving the performance of Single Molecule Localization Microscopy (SMLM) by measuring the 3D position and orientation of fluorophores. Orientation parameters provide information about protein organization and structure that is inaccessible by pure localization techniques. By modulating the fluorescence in the pupil plane, it is possible to encode 3D information in the shape of the Point Spread Function (PSF) of the system.

In this work we show that the separation of the fluorescence on the circular basis (LHC/RHC), in contrast with the more common separation into two linear polarization components, is advantageous: the PSFs are identical in both channels, their shape only depends on the out-of-plane angle, and they rotate as a function of the azimuthal angle. The z-coordinate can be deduced by the relative angle between the two PSFs. Finally, we compare several techniques: one using only the circular polarization splitting, and two using additionally polarization dependent phase masks: stressed-engineered optic,[1] and S-plate [2]).

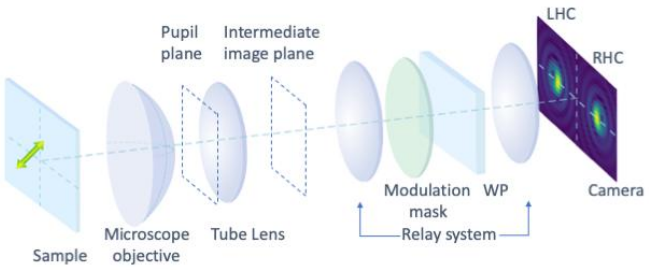

Fig. 1: Schematics of the setup for PSF engineering**.**

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