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How to assess depth-of-field extension strategies applied to single-molecule super-resolution microscopes?

Olivier Lévêque^{1,*}, Caroline Kulcsár¹, Antony Lee^{2,3}, Pierre Bon^{2,3},
Laurent Cognet^{2,3} and François Goudail¹

¹Université Paris-Saclay, Institut d'Optique Graduate School, CNRS, Laboratoire Charles Fabry, 91127, Palaiseau, France.

²Université de Bordeaux, Laboratoire Photonique Numérique et Nanosciences, UMR 5298, 33400, Talence, France.

³Institut d'Optique & CNRS, LP2N UMR 5298, 33400, Talence, France.

*olivier.leveque@institutoptique.fr

Abstract: Phase masks used to extend the depth-of-field (DoF) of localization microscopes are often designed to increase localization accuracy despite a loss in detection probability. We propose a method to optimize DoF extended single-molecule localization techniques taking into account the trade-off between detection probability and localization accuracy. © 2021 The Author(s)

1. Context

Super-resolution microscopy resolves biological subcellular structures beyond the diffraction limit. For instance, using photo-activated localization microscopy (PALM), lateral resolution of 2 to ~ 25 nm has been achieved by localizing individual fluorophore positions [1]. To maximize lateral resolution, the microscope objective must have very high numerical aperture, which considerably limits the depth-of-field (DoF) of this imaging technique and therefore the depth of exploration with which a biological sample can be probed. Indeed, in many applications, the region of interest may not be confined within the natural DoF range of the microscope objective and it is necessary to extend the DoF of the imaging system beyond this limit.

2. Extending depth-of-field using a phase mask

Several works have demonstrated that placing a well-designed phase mask in the Fourier plane of a localization microscope can significantly increase its DoF [2, 3]. We have recently proposed an easy-to-manufacture annular binary phase mask to improve the 2D-localization performance over a wide defocus range [4]. This type of mask is based on concentric rings implementing phase modulation of alternatively 0 and π radians at a nominal wavelength, and is optimized according to the Cramér-Rao bound (CRB) as a localization precision criterion. We have represented, on the top of Fig. 1.a, the variation with defocus of the point spread function (PSF) profile of a microscope equipped with this mask. In this graph, the parameter ψ represents the optical path difference between the focused and defocused spherical wavefronts at the edge of the pupil [5, 6] and is used as a reduced parameter to quantify defocus. We observe that this mask modifies the PSF in such a way that the lateral localization precision becomes quasi-invariant along the optical axis. This is confirmed by Fig. 1.b, which displays the normalized 2D-localization performances of a microscope without mask (blue dotted line) and with this phase mask (red dashed line). It is observed that using this mask significantly improves the localization of out-of-focus fluorophores to the expense of a loss of localization precision in the focal plane (e.i. $\psi = 0$).

3. Trade-off between detection probability and localization accuracy

However, the presence of a phase mask provokes a spreading of the PSF, which strongly affects the fluorophore pre-detection step that always precedes application of the super-localization estimator. We quantify the loss of detection probability induced by an optimal phase mask with the detection signal-to-noise ratio, that we denote SNR_Q . The pre-detection capacity is an increasing function of this parameter. We have represented the SNR_Q as a function of the defocus parameter in Fig. 1.c. The red dashed line corresponds to the mask optimized with the localization criterion. We notice that the SNR_Q value drops for $\psi = 0$. In other words, the probability of detection of fluorophore in the focal plane decreases drastically by using a phase mask optimized with a localization criterion. Hence, when optimizing a DoF extending phase mask, it is of utmost importance to take into account probability

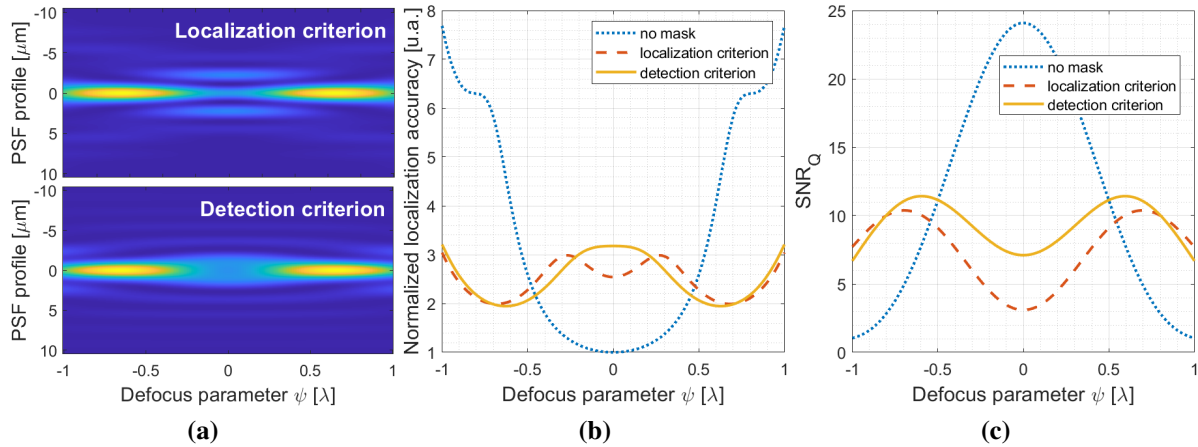


Fig. 1. **(a)** Variation of the transverse profile of the PSF as a function of the defocus when the microscope is equipped with a mask optimized with localization criterion or detection criterion. **(b)** 2D-localization accuracy without mask (blue dotted line), with optimized mask based on localization criterion (red dashed line) and with optimized mask based on detection criterion (yellow solid line) as a function of defocus. **(c)** Detection criterion evaluated for the same optical systems as a function of defocus.

of detection as well as localization precision. We have developed a methodology based on the SNR_Q to optimize DoF extended localization techniques according to the trade-off between detection probability and localization accuracy. An example of result is displayed in Fig. 1 by the yellow solid lines. It shows that this new detection criterion gives rise to phase masks with better probability of detection while preserving localization precision. This methodology also allows us to perform a quantitative comparison, in terms of both localization precision and detection capacity, between the phase masks previously developed in the literature [2, 3, 7] and the binary annular phase masks recently proposed by our team [8].

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