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On the equivalence of binary phase masks optimized for localization or detection in extended depth-of-field localization microscopy

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Abstract: Binary annular masks have recently been proposed to extend the depth of field (DoF) of single-molecule localization microscopy (SMLM). A strategy for designing optimal masks has been introduced based on maximizing the emitter localization accuracy, expressed in terms of Fisher information, over a targeted DoF range. However the complete post-processing pipeline to localize a single emitter consists of two successive steps: the *detection*, where the regions containing emitters are determined, and the *localization*, where the sub-pixel position of each detected emitter is estimated. Phase masks usually optimize only this second step. The presence of a phase mask also affecting the detection, the purpose of this article is to quantify and mitigate this effect. Using a rigorous framework built from a detection-oriented information theoretical criterion (Bhattacharyya distance), we demonstrate that in most cases of practical significance, annular binary phase masks maximizing Fisher information do also maximize the detection probability. This result supports the common design practice consisting of optimizing a phase mask by maximizing the Fisher information only.

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1. Introduction

Over the past few decades, single-molecule localization microscopy (SMLM) and single particle tracking (SPT) have become indispensable tools for studying the structure and dynamics of biological samples with nanometer resolution [1]. Both approaches conceptually share the same principle: they rely on imaging sequentially single molecules that are sufficiently far away from each other to be optically resolved at the diffraction limit, allowing their individual localization to be determined at nanometer resolution in each image. In SMLM, one observes distinct photo-activatable or blinking molecules in each image of the sequence, whereas in SPT, the same diffusing molecule is tracked through the image sequence. In both modalities, the set of all localizations collected in the image sequence provides structural or dynamic information about the sample under study.

Standard methods localize the molecule in 2D within the imaging plane. However, it is also possible to measure its 3D localization. This can be achieved using spatio-temporally modulated illumination and camera [2] or single pixel imaging [3]. It is also possible to insert a phase mask in the Fourier plane of the microscope [4], or a diffraction grating just before the imaging plane [5], in order to modify the detected point spread function (PSF) in a non-symmetric manner along the optical axis with respect to the imaging plane. Such PSF engineering methods have

45 been shown to be very efficient to estimate the 3D position of fluorescent emitters within the
46 natural depth of field (DoF) range of the microscope [5, 6]. However, in SMLM and SPT, high
47 numerical aperture (NA) is commonly used to maximize the collection efficiency of the photons
48 emitted by weak single molecule emitters, and to ensure high lateral localization precision. The
49 counterpart is that high NA objectives strongly limit the depth of field (DoF) of the acquired
50 images. Several developments have thus aimed at extending the DoF range of SMLM and SPT
51 microscopes. Two examples of commonly used masks that achieve extended DoF (EDoF) in
52 3D are the double-helix [7] and the tetrapod [8] masks. The penalty for using such masks is a
53 consequent broadening of the resulting PSFs which thus requires the use of bright emitters or
54 particles for proper super-localization.

55 In consequence, extending the DoF without trying to localize emitters along the optical axis
56 (*i.e.*, to localize them in 2D along an extended DoF range) can be useful in some applications,
57 since doing so, projection of thick 3D volumes on a 2D plane can be obtained at nanometer
58 resolution [9]. This can be particularly useful in SPT applications that aim to analyze single
59 particle trajectories, such as, for instance, deep-tissue high speed SPT where the number of
60 photons might be too low to apply 3D localization techniques.

61 For those applications where the depth of the emitter is not estimated using a PSF engineering
62 method, we have recently proposed an optimized annular binary phase mask that extends the
63 microscope DoF range [10] (see Fig. 1). The optimization criterion is based on the Cramér-Rao
64 bound (CRB), which defines the theoretical limit for localization accuracy of a fluorescent emitter
65 in the imaging plane. We have shown that using a mask that minimizes the largest value of the
66 CRB over the targeted EDoF range, and applying an adapted post-processing algorithm, makes
67 it possible to generate excellent 2D super-localization accuracy throughout the desired imaged
68 volume, at the cost of a moderate loss of localization accuracy in the focal plane as compared to
69 the situation where no mask would be used [10]. The effectiveness of these masks was recently
70 demonstrated in a SPT experiment [11].

71 However, it has to be kept in mind that the complete post-processing pipeline to localize a single
72 emitter in an image consists of two successive steps: the *pre-localization* (also called *detection*),
73 where the regions of the image containing emitters are determined, and the *localization*, where the
74 sub-pixel position of each detected emitter is estimated [12]. Phase masks are usually optimized
75 by taking into account only this second step. However, it is clear that the presence of a phase
76 mask also affects the detection step as well. It is therefore important to quantify the effect that the
77 insertion of a phase mask optimized for localization in the microscope produces on the detection
78 step. This is the question addressed in this article, which is organized as follows. Section 2
79 defines the imaging model, the microscope DoF range, and the phase mask architecture we use.
80 The pre-localization step of a fluorescent emitter is then formalized in Section 3 as a classification
81 problem. To quantify the effect of a phase mask on detection performance, we propose in
82 Section 4 a detectability metric based on the Bhattacharyya distance [13]. This metric is then
83 used in Section 5 to optimize phase masks for detection over a prescribed DoF range. In Section 6,
84 these optimal masks are compared to the masks that were optimized for localization-only in a
85 previous study [14]. Section 7 is devoted to concluding remarks and perspectives.

86 2. The depth of field (DoF) and its extension

87 We consider fluorescent emitters having nanometric size. They can be considered as point-like
88 light sources since their dimensions are not resolved by the microscope. Let us denote the
89 lateral position of an emitter in the sample by the coordinates $\theta = (x_p, y_p)$ and its longitudinal
90 position (along the optical axis) by the z_p coordinate. It is assumed that the microscope objective
91 is object-space telecentric, aplanatic (*i.e.*, it fulfills the Abbe sine condition), and limited by
92 diffraction over its field of view. As a consequence, the lateral magnification, denoted by M , does
93 not depend on the z_p longitudinal coordinate and the image of the emitter is spatially invariant.

94 The fluorescence signal distribution in the image plane (on the sensor), at coordinates (x, y) , is
 95 therefore proportional to:

$$f_{\psi}(x, y, \theta) \propto \left| \int_0^1 J_0 \left(\frac{2\pi \text{NA} r}{\lambda |M|} \sqrt{(x - |M|x_p)^2 + (y - |M|y_p)^2} \right) e^{i\Phi_{\psi}(r)} r \, dr \right|^2 \quad (1)$$

96 with $\iint f_{\psi}(x, y, \theta) \, dx \, dy = 1$ and where $J_0(\cdot)$ is the Bessel function of the first kind of order 0, λ
 97 is the wavelength of the collected light and NA is the object numerical aperture.

98 The pupil phase function $\Phi_{\psi}(r)$, involved in Eq. (1), characterizes the phase difference in the
 99 exit pupil between a real aberrant wavefront and a spherical reference surface. In the presence of
 100 defocus aberration, the function $\Phi_{\psi}(r)$ is classically described by a quadratic function of the
 101 reduced radial pupil coordinate r [15]:

$$\Phi_{\psi}(r) \simeq \frac{2\pi}{\lambda} \psi r^2 \quad (2)$$

102 where ψ is the defocus parameter which characterizes the phase deviation $2\pi\psi/\lambda$ at the pupil
 103 edge (*i.e.*, when $r = 1$) in the presence of defocus aberration. The parameter ψ has the following
 104 expression [16]:

$$\psi \simeq \frac{\text{NA}^2}{2n} (z_p - z_{\text{focus}}) \quad (3)$$

105 where n is the matched refractive index of the sample and z_{focus} the position at which the
 106 instrument is focused.

107 When the optical system is perfectly focused on the fluorescent emitter to be imaged (*i.e.*,
 108 $z_{\text{focus}} = z_p$), there is no defocus aberration. The value of the defocus parameter ψ is then equal to
 109 0, which cancels the pupil phase function $\Phi_{\psi}(r)$ defined in Eq. (2). The PSF defined in Eq. (1)
 110 is then described by the Airy spot:

$$f_{\text{Airy}}(x, y, \theta) \propto \left| \frac{J_1 \left(\frac{2\pi \text{NA}}{\lambda |M|} \sqrt{(x - |M|x_p)^2 + (y - |M|y_p)^2} \right)}{\frac{2\pi \text{NA}}{\lambda |M|} \sqrt{(x - |M|x_p)^2 + (y - |M|y_p)^2}} \right|^2 \quad (4)$$

111 where J_1 denotes the Bessel function of the first kind of order 1.

112 In the presence of defocus aberration (*i.e.*, $|\psi| > 0$), the PSF of the imaging system degrades.
 113 If the Rayleigh criterion is respected (*i.e.*, $|\psi| \leq \lambda/4$), the central value of the PSF decreases by
 114 more than 20% with an almost negligible deformation of the Airy spot. It is therefore common
 115 to define the natural DoF of the microscope by the interval $\psi \in [-\lambda/4, \lambda/4]$.

116 To extend the DoF of an imaging system without changing its aperture, a phase mask can
 117 be placed in its exit pupil [17]. The mask introduces a phase modulation in the aperture stop
 118 that modifies the PSF so that it becomes less sensitive to defocus aberration. Several mask
 119 architectures can be used. In this paper, we consider annular binary phase masks which have the
 120 advantage of being easy to manufacture while having a DoF extension capacity equivalent to that
 121 of masks whose phase varies continuously [18]. These masks are composed of concentric rings,
 122 as shown in Fig. 1. A mask with L rings and unit aperture radius is defined by the set of its $L - 1$
 123 radii represented by the vector $\boldsymbol{\rho} = (\rho_1, \dots, \rho_{L-1})$. Each ring adds to the incident wavefront a
 124 phase of 0 or π radians at the nominal wavelength λ of the incident light. Thus, in the presence
 125 of defocus aberration and a phase mask, the pupil phase function defined in Eq. (2) becomes:

$$\Phi_{\psi}(r) \simeq \frac{2\pi}{\lambda} \psi r^2 + \Phi_{\text{mask}}(r, \boldsymbol{\rho}) \quad (5)$$

126 where $\Phi_{\text{mask}}(r, \boldsymbol{\rho})$ denotes the binary phase function of the mask (0 or π radians) and $\boldsymbol{\rho}$ its
 127 parameter vector.

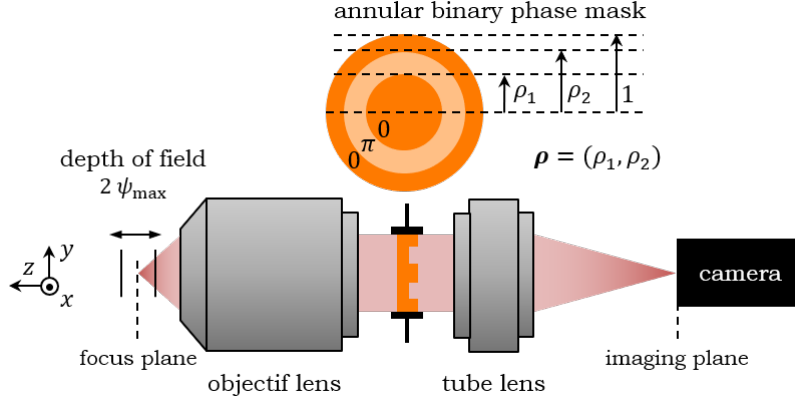


Fig. 1. Schematic diagram of an optical microscope whose its DoF has been extended by placing an optimized annular binary phase mask in the aperture stop of its objective.

128 3. Statistical hypothesis testing

129 The detection of a fluorescent emitter can be formulated as a classification problem, where
 130 the acquired data are automatically distinguished according to whether or not they contain
 131 a fluorescence signal from an emitter. For that purpose, let us consider a thumbnail of size
 132 $(2P + 1) \times (2P + 1)$ pixels and define the two following hypotheses.

133 **Hypothesis H1:** One emitter (and only one) is present in the thumbnail. The signal measured
 134 at pixel (i, j) is denoted by s_{ij} and consists of the fluorescence signal from the emitter and of
 135 a spatially constant background of mean b coming from *e.g.* from the electronic offset of the
 136 sensor or from the autofluorescence of the biological medium. Since both signals are disturbed
 137 by shot noise, the discrete random variable s_{ij} under the hypothesis H1 is a Poisson distribution:

$$P_{1,ij}[k] = \frac{\lambda_{1,ij}^k}{k!} \exp(-\lambda_{1,ij}) \quad (6)$$

138 with mean equal to

$$\lambda_{1,ij} = N_0 \mu_{ij}^{\psi, \theta} + b . \quad (7)$$

139 In this equation, N_0 denotes the average number of photo-electrons measured from the emitter
 140 and $\mu_{ij}^{\psi, \theta}$ corresponds to the value of the imaging system PSF, defined in Eq. (1), integrated over
 141 the square pixel (i, j) of side length Δ_{xy} :

$$\mu_{ij}^{\psi, \theta} = \int_{(i-\frac{1}{2})\Delta_{xy}}^{(i+\frac{1}{2})\Delta_{xy}} \int_{(j-\frac{1}{2})\Delta_{xy}}^{(j+\frac{1}{2})\Delta_{xy}} f_{\psi}(x, y, \theta) dx dy . \quad (8)$$

142 **Hypothesis H0:** There is no fluorophore in the thumbnail; only the background signal is
 143 measured. The probability distribution of the discrete random variable s_{ij} under this hypothesis
 144 is denoted by $P_0[k]$. It is again a Poisson distribution, as defined in Eq. (6), but with a mean
 145 independent of the pixel (i, j) :

$$\lambda_0 = b . \quad (9)$$

146 Figure 2 illustrates these two hypotheses by a set of simulated thumbnails in which an emitter
 147 is either absent [see Fig. 2(a)] or present [see Figs. 2(b-d)] with three different values of the

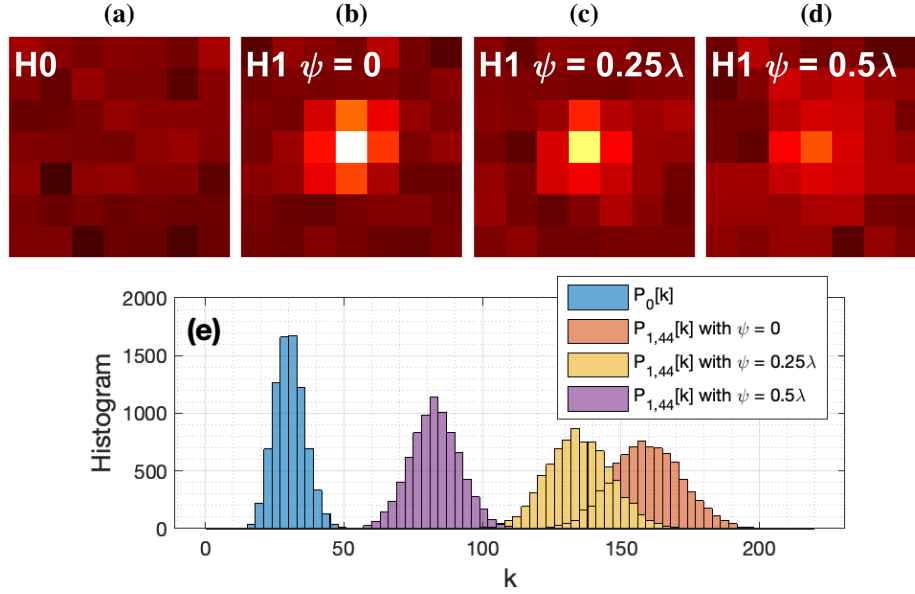


Fig. 2. (a-d) Simulations of thumbnails \underline{s} verifying the H0 or H1 hypothesis for optical microscopes without phase mask and for several values of the defocus parameter ψ . (e) Histogram of the value of the central pixel $(i, j) = (4, 4)$ made on 8 000 noise realizations. The microscope configuration and the simulation parameters are defined in Table 1 with $N_0 = 500 \text{ ph.e}^-$ and $b = 30 \text{ ph.e}^-$.

148 defocus parameter ψ , namely $\psi = \{0, 0.25\lambda, 0.5\lambda\}$. Figure 2(e) represents the histogram of
 149 the central pixel value constructed from 8 000 different noise realizations. These numerical
 150 simulations are carried out with the parameters described in Table 1. It is clearly seen that as the
 151 defocus parameter ψ increases, the PSF degrades: it spreads and disappears in the background
 152 fluorescence. With the naked eye, comparing Fig. 2(d) with Fig. 2(a), it is clear that it will become
 153 increasingly difficult to determine whether a fluorophore is present or not. This is also seen in
 154 Fig. 2(e), where the histogram associated with the central pixel $P_{1,44}[k]$ approaches $P_0[k]$ as ψ
 155 increases: the hypotheses H1 and H0 will “merge” and become less and less discernible.

156 A decision algorithm consists in automatically classifying the acquired data sets $\underline{s} =$
 157 $(s_{ij})_{(i,j) \in [-P,P]^2}$ by distinguishing those containing a fluorescence signal (*i.e.*, verifying the
 158 hypothesis H1) and those that are empty (*i.e.*, verifying the hypothesis H0). Neyman *et al.*
 159 showed in 1933 that the optimal algorithm — *i.e.*, the one that gives the maximal detection
 160 probability for a given probability of false alarm — consists in comparing the likelihood ratio to
 161 a threshold [19].

162 4. Bhattacharyya distance

163 No hypothesis test, even the optimal one like Neyman *et al.*'s, can lead to a perfect classification.
 164 Moreover, as shown in Fig. 2, it is expected that the farther a fluorophore is from the focal
 165 plane (*i.e.*, $|\psi|$ large), the less discernible the H1 and H0 hypotheses, and the more difficult the
 166 classification problem. In order to evaluate the performance of a hypothesis testing algorithm,
 167 we can compute its error probability, that is, the probability that a thumbnail \underline{s} is assigned to the
 168 wrong class.

169 The Bayes error rate ϵ is the smallest possible error probability that a classification algorithm
 170 can lead to [19]. Since under both hypotheses H1 and H0 the measured pixel values s_{ij} are

Simulation parameters	Symbols	Values
Fluorophore position in the plane	(x_p, y_p)	$(0, 0) \mu\text{m}$
Fluorophore wavelength emission	λ	700 nm
PSF image length	$2P + 1$	21 pixels
Pixel length	Δ_{xy}	$10 \mu\text{m}$
Object numerical aperture	NA	1.3
Lateral magnification	M	60

Table 1. Microscope configuration and simulation parameters used.

171 statistically independent of each other, the expression of ϵ depends on $P_{1,ij}[k]$ and $P_0[k]$ as
 172 follows [20]:

$$\epsilon = \sum_{s_{11}, s_{12}, s_{21}, \dots} \min \left\{ p_1 \prod_{i=-P}^P \prod_{j=-P}^P P_{1,ij}[s_{ij}]; p_0 \prod_{i=-P}^P \prod_{j=-P}^P P_0[s_{ij}] \right\} \quad (10)$$

173 with p_1 and p_0 the prior probabilities of the hypotheses H1 and H0. The sum is performed on
 174 all possible values of the $(2P + 1)^2$ pixels, so that $s_{ij} = \{0, 1, \dots, +\infty\}$. Using the inequality
 175 $\min\{a; b\} \leq a^\gamma b^{1-\gamma}$ (where $a, b \geq 0$ and $0 \leq \gamma \leq 1$), it is easily seen that Eq. (10) can be upper
 176 bounded by:

$$\epsilon_u(\gamma) = \exp \left[- \sum_{i=-P}^P \sum_{j=-P}^P C_{ij}(\gamma) \right] \quad 0 \leq \gamma \leq 1 \quad (11)$$

177 where

$$C_{ij}(\gamma) = - \ln \left[\sum_{k=0}^{+\infty} P_{1,ij}[k]^\gamma P_0[k]^{1-\gamma} \right] \quad (12)$$

178 is the Chernoff distance between the probability laws $P_{1,ij}[k]$ and $P_0[k]$ [21]. Since these laws
 179 follow Poisson distributions of means $\lambda_{1,ij}$ and λ_0 respectively, as defined in Eqs. (7) and (9), the
 180 Chernoff distance has the following closed-form expression:

$$C_{ij}(\gamma) = \gamma \lambda_{1,ij} + (1 - \gamma) \lambda_0 - \lambda_{1,ij}^\gamma \lambda_0^{1-\gamma}. \quad (13)$$

181 Thus, the smallest upper bound on ϵ (also called the Chernoff bound) is obtained with the value
 182 of γ that maximizes the sum of $C_{ij}(\gamma)$. This optimal value depends on the means $\lambda_{1,ij}$ and λ_0
 183 and has no closed-form expression. However, it can be shown that for many applications, the
 184 optimal value of γ is close to 0.5 [22]. This is what can be observed in Fig. 3, where the optimal
 185 value of the parameter γ is plotted as a function of the defocus parameter ψ for data simulated
 186 with the parameters defined in Table 1: for all the considered values of ψ , the Chernoff bound is
 187 reached for a value of γ close to 0.5.

188 Thus, $\epsilon_u(0.5)$ is a good candidate to characterize the detectability of a fluorophore since it
 189 allows to approach the Chernoff bound by a closed-form expression. This metric, which is called
 190 the Bhattacharyya distance [13], has the following expression:

$$\mathcal{B}(\rho, \psi) = \sum_{i=-P}^P \sum_{j=-P}^P C_{ij}(0.5) = \frac{b}{2} \sum_{i=-P}^P \sum_{j=-P}^P \left(\sqrt{\frac{N_0}{b} \mu_{ij}^{\psi, \theta} + 1} - 1 \right)^2 \quad (14)$$

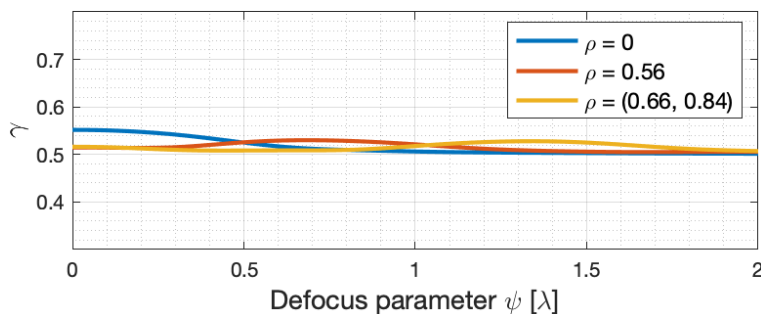


Fig. 3. Optimal value of the parameter γ , which minimizes the upper bound of the Bayes error rate $\epsilon_u(\gamma)$. This value is plotted as a function of the defocus parameter ψ for different mask parameters ρ . The microscope configuration and the simulation parameters used are defined in Table 1 with $N_0 = 500 \text{ ph.e}^-$ and $b = 30 \text{ ph.e}^-$.

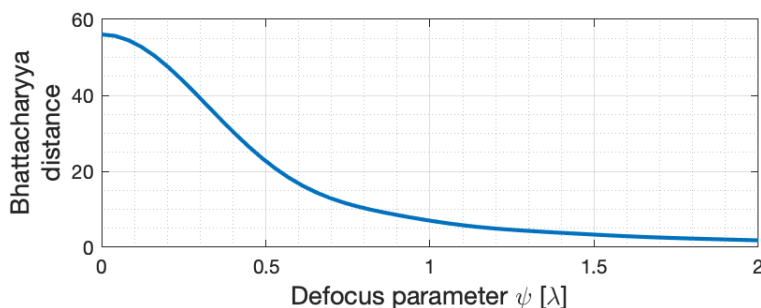


Fig. 4. Evolution of the Bhattacharyya distance for an optical system without phase mask ($\rho = 0$) as a function of the defocus parameter ψ . The microscope configuration and the simulation parameters used are defined in Table 1 with $N_0 = 500 \text{ ph.e}^-$ and $b = 30 \text{ ph.e}^-$.

191 where we have explicitly indicated its dependence on the mask parameter vector ρ and on the
 192 defocus parameter ψ . It allows us to evaluate the intrinsic difficulty of a detection problem in the
 193 presence of spatially uncorrelated Poisson noise. The interest of using Eq. (14) as a measure of
 194 contrast in imaging science has been illustrated in Refs. [22] and [23].

195 The Bhattacharyya distance \mathcal{B} can be interpreted as a measure of similarity (or separability)
 196 between the hypotheses H_0 and H_1 . It will therefore be used to evaluate the intrinsic ability
 197 with which the fluorescence signal of an emitter can be detected in a measured image. As an
 198 illustration, Fig. 4 represents the evolution of the distance \mathcal{B} in Eq. (14) for an optical system
 199 without phase mask ($\rho = 0$) as a function of the defocus parameter ψ . We observe that it
 200 decreases as ψ increases. In other words, the hypotheses H_1 and H_0 are less and less discernible.
 201 This logical result quantifies the fact that a fluorophore far from the focal plane ($|\psi| > 0$) is more
 202 difficult to detect than when it is located in the focal plane ($\psi = 0$).

203 5. Phase mask optimization for detection applications

204 To extend the depth over which fluorescent emitters can be detected, we propose to optimize
 205 annular binary phase masks using the criterion based on the Bhattacharyya distance defined in
 206 Eq. (14). A reasonable criterion for mask optimization is to maximize the smallest value of
 207 the Bhattacharyya distance within the targeted EDoF range $[-\psi_{\max}, \psi_{\max}]$. The optimal mask
 208 parameters, denoted by the vector ρ_{opt} , are thus obtained by solving the following optimization

209 problem:

$$\boldsymbol{\rho}_{\text{opt}} = \arg \max_{\boldsymbol{\rho}} J_{\text{det}}(\boldsymbol{\rho}) \quad \text{with} \quad J_{\text{det}}(\boldsymbol{\rho}) = \min_{\psi \in [-\psi_{\text{max}}, \psi_{\text{max}}]} \mathcal{B}(\boldsymbol{\rho}, \psi) . \quad (15)$$

210 Given the expression of \mathcal{B} in Eq. (14), the optimal mask parameters $\boldsymbol{\rho}_{\text{opt}}$ depend on the
 211 experimental conditions through the ratio N_0/b , which will be called the *photon balance* in
 212 the following. Since the cost function $J_{\text{det}}(\boldsymbol{\rho})$ is highly non-convex, Eq. (15) is solved using
 213 the particle swarm optimization algorithm [24]. This approach relies on the collaboration of
 214 individuals. Based on simple displacement rules, a set of particles (whose coordinates describe
 215 the radii of the rings of a binary annular mask) explore the optimization landscape and gradually
 216 converge together towards a local minimum. Since there is no guarantee of finding the global
 217 minimum, different runs may converge to different optimal masks with similar performance.
 218 The optimization is performed with ring structures composed of a maximum of $L = 5$ rings.
 219 However, considering the optical parameters of Table 1 and N_0/b values belonging to the interval
 220 $[8, 500]$, we observed that the mask performance stabilizes from a number lower than 5 rings.
 221 The optimal mask parameters $\boldsymbol{\rho}_{\text{opt}}$ also depend on the lateral emitter position with respect to the
 222 pixel grid. However, one can show by simulation that this position has very little influence on the
 223 optimal mask parameters $\boldsymbol{\rho}_{\text{opt}}$. To facilitate the comparison between the masks that are optimal
 224 for detection and those optimal for localization obtained in Ref. [14], we will assume that the
 225 emitter is located at the center of a pixel so that $\boldsymbol{\theta}_0 = (0, 0)$.

226 Figure 5(a) plots the optimal mask parameters as a function of the photon balance N_0/b for
 227 three different targeted DoF ranges: $\psi_{\text{max}} = \{1\lambda, 1.5\lambda, 2\lambda\}$. For a given value of N_0/b , the mask
 228 parameters are represented by a vertical set of points: each point characterizes the radius ρ_ℓ of a
 229 ring with $\ell \in \{1, \dots, L-1\}$ (radii equal to 0 or 1 are not represented). The performance of the
 230 masks is displayed using solid markers (associated with the legend “Detection”) in Fig. 5(b) for
 231 $\psi_{\text{max}} = 1\lambda$, in Fig. 5(c) for $\psi_{\text{max}} = 1.5\lambda$ and in Fig. 5(d) for $\psi_{\text{max}} = 2\lambda$. In these figures, the mask
 232 performance is normalized by dividing the optimal value of the criterion $J_{\text{det}}(\boldsymbol{\rho}_{\text{opt}})$ by $J_{\text{det}}(0)$
 233 the value of the criterion obtained without a mask. We will comment in detail the curves with
 234 hollow markers in the next paragraph.

235 Let us first analyze the results obtained for a targeted DoF range of $\psi_{\text{max}} = 1\lambda$. In Fig. 5(a)
 236 (solid blue round markers), it is observed that whatever the value of N_0/b , the optimal masks are
 237 composed of only $L = 2$ rings. When $N_0/b < 200$, the parameter ρ_1 of the optimal mask increases
 238 almost linearly from 0.56 to 0.66. It then stabilizes at $\rho_1 = 0.68$ when $N_0/b \in [200, 450]$, and
 239 then when $N_0/b > 450$, ρ_1 is zero: the optimal configuration corresponds to the absence of a
 240 mask. Moreover, we observe in Fig. 5(b) (solid blue round markers) that the performance gap
 241 between the cases with and without mask is larger for small values of N_0/b . For example, when
 242 $N_0/b = 8$, we obtain $J_{\text{det}}(\boldsymbol{\rho}_{\text{opt}}) = 1.78 \times J_{\text{det}}(0)$.

243 The use of a phase mask therefore significantly improves the detection of fluorophores. Small
 244 values of N_0/b correspond to situations where the dominant source of noise is due to background
 245 fluctuations (we shall call this situation *Scenario B*, as in Ref. [14]). In this scenario, the PSF
 246 of the imaging system is drowned in the background noise when no mask is used. The optimal
 247 mask allows the PSF to be modified in order to improve the contrast between the fluorophore
 248 image and the background.

249 We also observe that $J_{\text{det}}(\boldsymbol{\rho}_{\text{opt}})$ tends asymptotically to $J_{\text{det}}(0)$ as the photon balance N_0/b
 250 increases, that is, when the dominant source of noise is the shot noise due to the fluorescence
 251 signal (this situation is called *Scenario A* in Ref. [14]). It means that in this case, the use of a
 252 phase mask does not improve nor deteriorate fluorophore detection: the contrast between the
 253 fluorophore and the background is sufficient for a correct detection over the DoF range $\psi_{\text{max}} = 1\lambda$
 254 without the help of a mask.

255 To explain this fact, we can notice that if the fluorescence signal is such that $N_0\mu_{ij}^{\psi, \theta_0} \gg b$

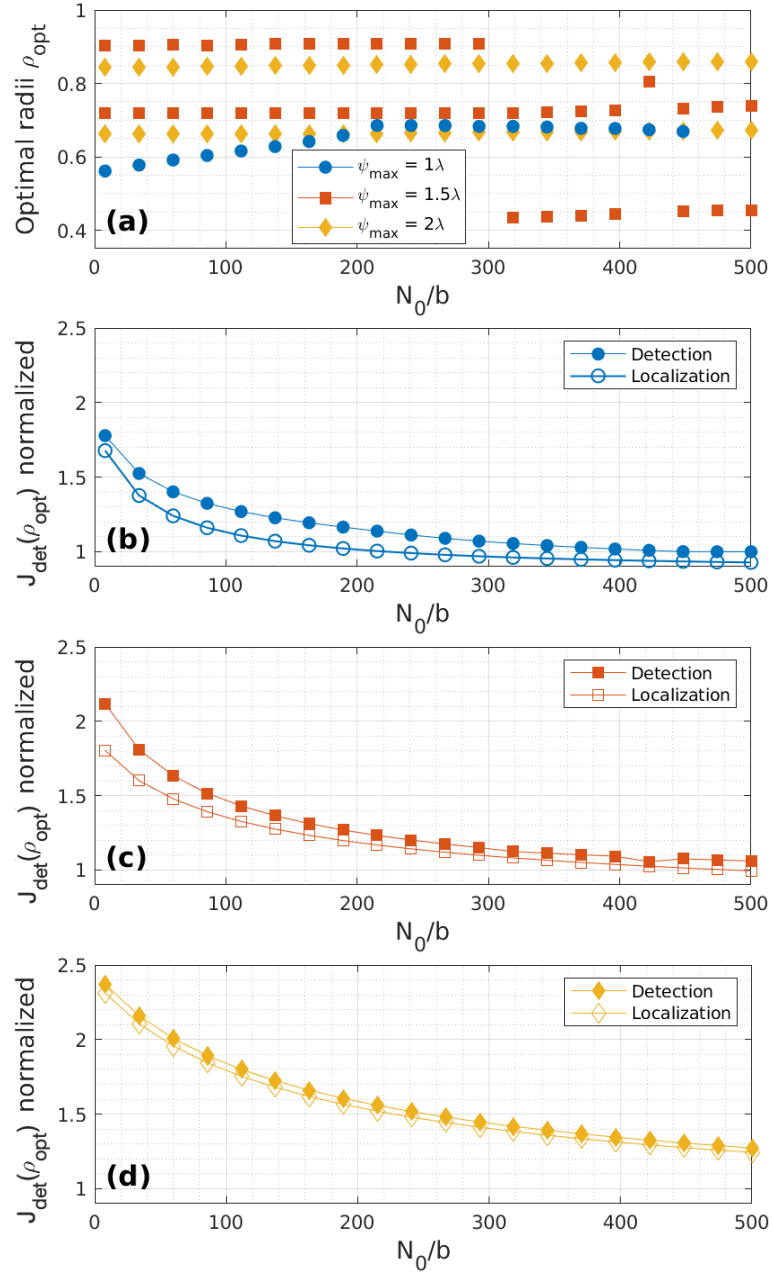


Fig. 5. (a) Evolution of the optimal annular binary phase mask parameters ρ_{opt} , defined in Eq. (15), as a function of the photon balance N_0/b with $\psi_{\text{max}} = \{1\lambda, 1.5\lambda, 2\lambda\}$. (b-d) Comparison of normalized detection criterion values, *i.e.*, $J_{\text{det}}(\rho_{\text{opt}})/J_{\text{det}}(0)$, respectively computed with optimal masks defined in Eqs. (15) and (20), for (b) $\psi_{\text{max}} = 1\lambda$, (c) 1.5λ and (d) 2λ . The microscope configuration and the simulation parameters used are defined in Table 1 with $N_0/b \in [8, 500]$.

256 (Scenario A), then the Bhattacharyya distance \mathcal{B} defined in Eq. (14) becomes:

$$\mathcal{B} \simeq \frac{N_0}{2} \sum_{i=-P}^P \sum_{j=-P}^P \mu_{ij}^{\psi, \theta_0}. \quad (16)$$

257 This approximate expression no longer depends on the parameters ρ and ψ since the conservation
258 of energy by a phase mask imposes:

$$\sum_{i=-P}^P \sum_{j=-P}^P \mu_{ij}^{\psi, \theta_0} = \text{constant}. \quad (17)$$

259 Thus, the criterion $J_{\text{det}}(\rho_{\text{opt}})$ defined in Eq. (15) tends asymptotically to $J_{\text{det}}(0)$.

260 As ψ_{max} increases, it is seen in Fig. 5(a) that the optimal masks are composed of a larger
261 number of rings: whereas for $\psi_{\text{max}} = 1\lambda$, the optimal mask consists of $L = 2$ rings, it is composed
262 of $L = 3$ rings for $\psi_{\text{max}} = 1.5\lambda$ or 2λ . This reflects the increasing difficulty of optimizing a phase
263 mask as the targeted DoF range widens. This result is similar to the one established for imaging or
264 localization, where the number of rings of the optimal mask also increases with ψ_{max} [10, 25].
265 Moreover, the conclusions previously established in Fig. 5(b) for the DoF range $\psi_{\text{max}} = 1\lambda$ remain
266 similar when $\psi_{\text{max}} = 1.5\lambda$ (see solid markers in Fig. 5(c)) or 2λ (see solid markers in Fig. 5(d)):
267 the performance gap between the cases with and without mask is maximum for $N_0/b = 8$ and
268 decreases as the photon balance increases. This means that $J_{\text{det}}(\rho_{\text{opt}})$ tends to $J_{\text{det}}(0)$ when N_0/b
269 increases.

270 We can also notice in Fig. 5(a) that as the targeted DoF range ψ_{max} widens, the optimal mask
271 parameters vary less and less as a function of the photon balance N_0/b . This result can be
272 explained by a conjecture established in Ref. [14] and observed in Figs. 2(b-d): for the same
273 value of N_0/b , the PSF spreads and drowns into the background noise as ψ_{max} increases. In
274 this case, the inequality $N_0 \mu_{ij}^{\psi, \theta_0} \ll b$ (Scenario B) is even more valid and the Bhattacharyya
275 distance \mathcal{B} is approximated by:

$$\mathcal{B}(\rho, \psi) \simeq \frac{1}{8} \frac{N_0^2}{b} \sum_{i=-P}^P \sum_{j=-P}^P \left(\mu_{ij}^{\psi, \theta_0} \right)^2, \quad (18)$$

276 and becomes proportional to the signal-to-noise ratio N_0^2/b . Thus, the optimal parameter vector
277 ρ_{opt} that minimizes the criterion $J_{\text{det}}(\rho)$ no longer depends on N_0/b . As a consequence, the
278 optimal masks shown in Fig. 5(a) vary less and less with the photon balance as ψ_{max} increases.

279 To conclude, the use of an optimal mask for detection applications is only necessary for
280 experimental conditions corresponding to the Scenario B (background noise dominant). For
281 other experimental conditions, the fluorophore image is sufficiently contrasted and the use of the
282 phase mask is no longer necessary for detection.

283 6. Comparison of masks optimal for detection and for localization

284 As mentioned in the Introduction, once the fluorescent emitter is detected, its position is finally
285 estimated with a sub-pixel resolution in the localization step.

286 In general, phase masks are never optimized for detection [8, 26]. For example, we have
287 optimized binary annular phase masks based solely on localization accuracy in Refs. [10] and [14].
288 In this case, the optimization criterion is based on the Fisher information matrix [20], which
289 quantifies the difficulty of estimating the lateral fluorophore position $\theta = (x_p, y_p)$, independently
290 of the (unbiased) algorithm used to perform this estimation. We have shown in Ref. [14] that
291 since the pixelated PSF described in Eq. (8) has almost circular symmetry, the non-diagonal

292 terms of the Fisher information matrix are negligible. Moreover, when $\theta_0 = (0, 0)$, the diagonal
 293 terms are strictly equal and reach their minima. In this case, the Fisher information matrix is
 294 then characterized by a single scalar value equal to:

$$\mathcal{I}(\boldsymbol{\rho}, \psi) = \sum_{i=-P}^P \sum_{j=-P}^P \frac{N_0^2 M^2}{N_0 \mu_{ij}^{\psi, \theta_0} + b} \left(\frac{\partial \mu_{ij}^{\psi, \theta_0}}{\partial x} \right)^2. \quad (19)$$

295 To improve the localization accuracy of fluorescent emitters over a targeted EDoF range, annular
 296 binary phase masks were optimized by solving the following problem:

$$\boldsymbol{\rho}_{\text{opt}} = \arg \max_{\boldsymbol{\rho}} J_{\text{loc}}(\boldsymbol{\rho}) \quad \text{with} \quad J_{\text{loc}}(\boldsymbol{\rho}) = \min_{\psi \in [-\psi_{\text{max}}, \psi_{\text{max}}]} \mathcal{I}(\boldsymbol{\rho}, \psi). \quad (20)$$

297 Optimal masks for Scenario A ($N_0 \mu_{ij}^{\psi, \theta_0} \gg b$) and Scenario B ($N_0 \mu_{ij}^{\psi, \theta_0} \ll b$) have been
 298 determined in Ref. [14].

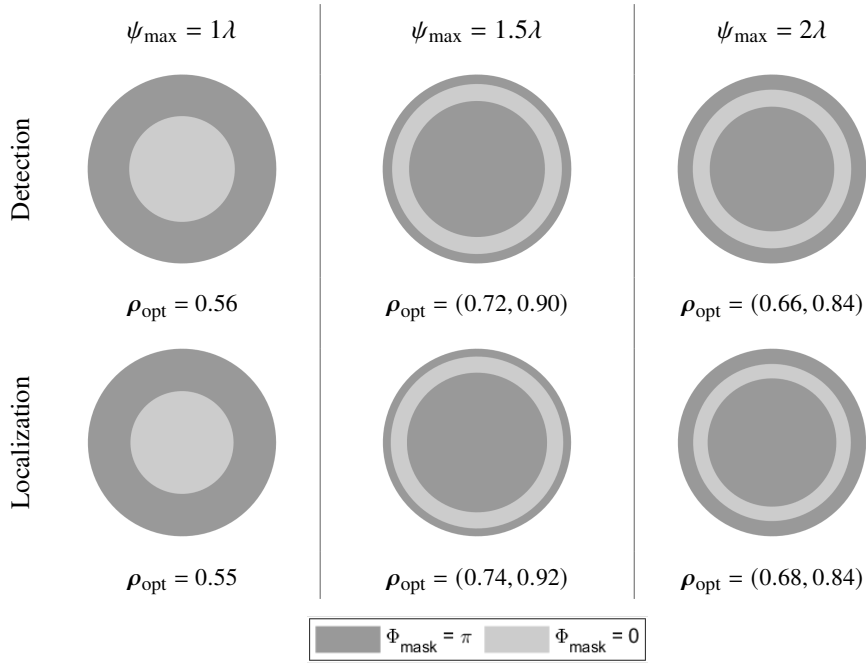


Table 2. Annular binary phase masks obtained by solving Eq. (15) [first row] and Eq. (20) [second row] for a EDoF range such that $\psi_{\text{max}} = \{1\lambda, 1.5\lambda, 2\lambda\}$. The simulation parameters are those defined in Table 1 with $N_0/b = 8$.

299 We have shown in Section 5 that the use of an optimal mask for detection is only necessary in
 300 experimental conditions corresponding to Scenario B, which corresponds to dominant background
 301 fluctuations. Thus, we compare in this section the annular binary phase masks optimal for
 302 detection and localization in this scenario. These masks are represented in Table 2, where the first
 303 row corresponds to the masks optimal for detection when $N_0/b = 8$, that have been represented
 304 in Fig. 5, and the second row corresponds to those optimized in Ref. [14] for localization under
 305 Scenario B.

306 We notice that the masks optimized for detection are similar to those optimized for localization.
 307 For example, when $\psi_{\text{max}} = 1$ the optimal mask for detection is composed of 2 rings with

308 $\rho_{\text{opt}} = 0.56$ while the one optimized for localization has also 2 rings and $\rho_{\text{opt}} = 0.55$. Furthermore,
 309 when $\psi_{\text{max}} = 1.5\lambda$, it is seen in Fig. 5(a) that the optimal mask for detection has 3 rings and
 310 $\rho_{\text{opt}} = (0.72, 0.90)$, while the one optimal for localization has also 3 rings with $\rho_{\text{opt}} = (0.74, 0.92)$.
 311 The same result is found when $\psi_{\text{max}} = 2\lambda$: the optimal mask for detection has $\rho_{\text{opt}} = (0.66, 0.84)$
 312 and the one optimal for localization $\rho_{\text{opt}} = (0.68, 0.84)$.

313 This remarkable similarity can be theoretically explained by analyzing the optimization criteria
 314 for detection and localization defined respectively in Eqs. (15) and (20) when the photon balance
 315 is small. Indeed, if we assume that $N_0\mu_{ij}^{\psi, \theta_0} \ll b$, these criteria can be respectively approximated
 316 by

$$J_{\text{det}}(\rho) \simeq \min_{\psi \in [-\psi_{\text{max}}, \psi_{\text{max}}]} \sum_{i=-P}^P \sum_{j=-P}^P \left(\mu_{ij}^{\psi, \theta_0} \right)^2 \quad (21)$$

317 and

$$J_{\text{loc}}(\rho) \simeq \min_{\psi \in [-\psi_{\text{max}}, \psi_{\text{max}}]} \sum_{i=-P}^P \sum_{j=-P}^P \left(\frac{\partial \mu_{ij}^{\psi, \theta_0}}{\partial x} \right)^2, \quad (22)$$

318 where the pixelated PSF verifies Eq. (17). We notice that maximizing the criteria given in
 319 Eqs. (21) and (22) under this constraint corresponds to searching for a phase mask producing
 320 PSFs as concentrated as possible on a small number of pixels.

321 To verify this conjecture, Figs. 6 and 7 display the cost functions $J_{\text{det}}(\rho)$ and $J_{\text{loc}}(\rho)$ respectively
 322 defined in Eqs. (15) and (20) as a function of the mask parameters ρ . The experimental conditions
 323 used for the simulation are those described in Table 1 with $N_0 = 500 \text{ ph.e}^-$ and $b = 30 \text{ ph.e}^-$ (*i.e.*,
 324 $N_0/b = 16.7$). Figure 6 represents the optimization landscapes for detection (solid blue line) and
 325 localization (dashed red line) of a 2-ring binary mask defined by its radius ρ_1 , for $\psi_{\text{max}} = 1\lambda$ and
 326 $N_0/b = 16.7$. We notice that these two optimization landscapes have similar shapes and reach
 327 their local extrema for almost the same parameter value ρ_1 . For $\psi_{\text{max}} = 1.5\lambda$ and $N_0/b = 16.7$,
 328 Fig. 7(a) represents the 3-ring mask optimization landscape for detection as a function of its radii
 329 (ρ_1, ρ_2) , and Fig. 7(b) is the optimization landscape for localization. We notice again that these
 330 two optimization landscapes have similar shapes and almost the same extrema — thus, almost
 331 the same optimal masks — that are indicated by a white cross.

332 The conditions of Scenario B (small number of useful photons and strong background), are the
 333 most demanding and frequently encountered in practice. We have shown that in these conditions,
 334 the EDoF masks optimized for detection and localization are identical. This result shows that
 335 the optimization method based on Fisher information to extend DoF that has been introduced in
 336 Ref. [10] optimizes both detection probability and localization accuracy.

337 In order to quantify the loss of detection performance that would result from using localization-
 338 optimized masks, we have plotted in Figs. 5(b)-5(d), with hollow markers, the normalized values
 339 of the detection criterion obtained by these masks, respectively for $\psi_{\text{max}} = \{1\lambda, 1.5\lambda, 2\lambda\}$, as
 340 a function of the photon balance N_0/b . We note that whatever the value of N_0/b , $J_{\text{det}}(\rho_{\text{opt}}^{\text{loc}})$
 341 (hollow markers) is always lower than $J_{\text{det}}(\rho_{\text{opt}}^{\text{det}})$ (solid markers) where $\rho_{\text{opt}}^{\text{loc}}$ refers to masks
 342 optimized for localization with Eq. (20) and $\rho_{\text{opt}}^{\text{det}}$ masks optimized for detection with Eq. (15).
 343 The difference between these two curves quantifies the relative loss (expressed as a fraction of
 344 $J_{\text{det}}(0)$) caused by the use of a mask optimized for localization. We can see that this relative loss
 345 does not exceed 17% when $\psi_{\text{max}} = 1\lambda$, 30% when $\psi_{\text{max}} = 1.5\lambda$ and 6% when $\psi_{\text{max}} = 2\lambda$. We
 346 therefore conclude that the masks optimized for localization only slightly degrade the probability
 347 of detection compared to the masks that optimize it.

348 7. Conclusion

349 We have investigated the DoF extension problem for single-molecule localization microscopy,
 350 focusing on the pre-localization step. After defining a rigorous framework built with an explicit

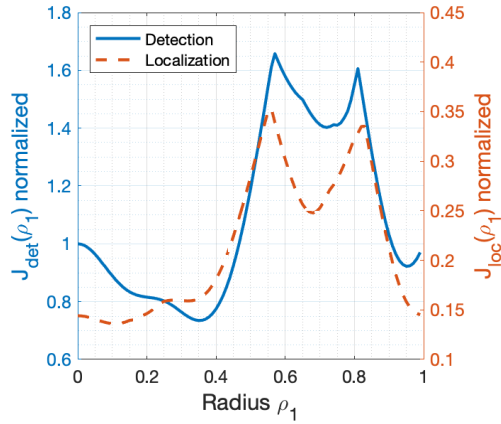


Fig. 6. Optimization landscapes of a 2-ring binary phase mask for detection (solid blue line) and localization (dashed red line) when $\psi_{\max} = 1\lambda$. The values of $J_{\text{det}}(\rho)$ defined in Eq. (15) and $J_{\text{loc}}(\rho)$ defined in Eq. (20) are normalized by dividing them by $J_{\text{det}}(0)$ and $I(0,0)$ respectively. The simulation parameters are given in Table 1 with $N_0 = 500 \text{ ph.e}^-$ and $b = 30 \text{ ph.e}^-$.

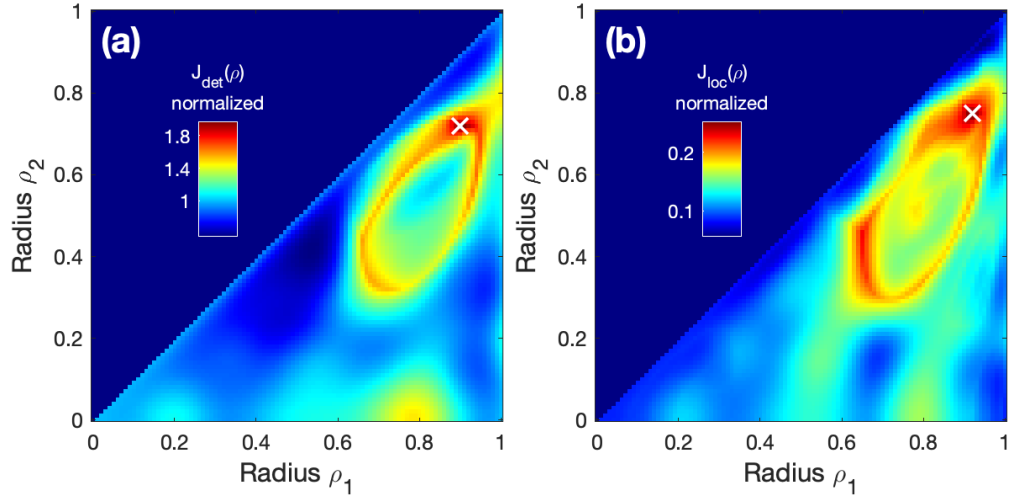


Fig. 7. Optimization landscapes of a 3-ring binary phase mask for (a) detection and (b) localization applications when $\psi_{\max} = 1.5\lambda$. The values of $J_{\text{det}}(\rho)$ defined in Eq. (15) and $J_{\text{loc}}(\rho)$ defined in Eq. (20) are normalized by dividing them by $J_{\text{det}}(0)$ and $I(0,0)$ respectively. The white cross corresponds to the global minimum of the criterion. $[\rho_1, \rho_2]$ are respectively equal to $[0.90, 0.72]$ for detection and $[0.92, 0.75]$ for localization. The simulation parameters are given in Table 1 with $N_0 = 500 \text{ ph.e}^-$ and $b = 30 \text{ ph.e}^-$.

351 and general information theoretical criterion to evaluate the detection probability of a fluorophore
352 in a thick imaged volume, we have shown that annular binary phase masks optimized using Fisher
353 information are adequate for both the detection probability and the localization accuracy. Indeed,
354 under the assumption of a low photon balance, the optimization criteria defined in Eqs. (15)
355 and (20) lead to similar optimal masks. Conversely, if the photon balance is large, the use of a
356 mask optimized for detection is not necessary and the one optimized for localization only slightly
357 degrades the detection probability. This result supports the common design practice consisting of
358 optimizing a phase mask with a criterion based solely on localization accuracy. In other words,
359 annular binary phase masks for DoF extension in single-molecule localization microscopy can be
360 optimized using only a criterion based on Fisher information.

361 Although the formalism used in this paper assumes that the masks have circular symmetry
362 (since they depend only on the radial coordinate r), the methodology developed can be easily
363 extended to masks without particular symmetry. An interesting perspective of this work will
364 therefore be to apply this methodology to other mask architectures, in order to verify the
365 generalization of the conclusions that we have reached for masks with annular binary phase.

366 **Disclosures.** The authors declare no conflicts of interest.

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