Tailoring the axial shape of the point spread function using the Toraldo concept

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Abstract: A novel procedure for shaping the axial component of the point spread function of nonparaxial focusing systems by use of phase-only pupil filters is presented. The procedure is based on the Toraldo technique for tailoring focused fields. The resulting pupil filters consist of a number of concentric annular zones with constant real transmittance. The number of zones and their widths can be adapted according to the shape requirements. Our method is applied to design filters that produce axial superresolution in confocal scanning systems.

OCIS codes: (100.6640) Superresolution; (110.1220) Apertures; (140.3300) Laser beam shaping; (170.1790) Confocal microscopy

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1. Introduction

Controlling the structure of the three-dimensional (3D) region that surrounds the focus of an optical system, i.e. the 3D point spread function (PSF), has long been the aim of many research efforts. In particular the design of techniques to overcome the limits in resolution imposed by diffraction in imaging systems is of great interest. Most of the reported apodization techniques have been aimed at improving the resolving capability of conventional two-dimensional (2D) imaging system; and therefore improving the transverse resolution [1-3]. However, when dealing with imaging systems designed to obtain the image of 3D samples, not only the transverse resolution but also the axial resolution is of great interest. In this context the design of pupil filters for obtaining axial superresolution was the purpose of many scientific contributions [3-6]. Another purpose was tailoring the whole 3D PSF [7-11]. Among all the techniques for designing pupil filters, one of the most innovative is the one reported by Toraldo di Francia [12]. Toraldo showed that by subdividing the pupil into the proper number of concentric annular zones with constant complex transmittance, a band-limited transverse diffraction pattern of any shape may be obtained. The Toraldo method has been further applied for shaping the transverse PSF [13-14], and also the axial PSF [15-17]. It should be noted however that in the later case the Toraldo method was not adapted to shape the axial PSF. On the contrary, the axial-shaping filters were obtained as a result of a comprehensive empirical study.

In this paper we present the axial form of Toraldo's pupil synthesis method. We show that by applying the Toraldo concept to a 1D properly nonlinearly mapped version of the pupil transmittance, one can control at will the positions of a finite number of zeros of the axial PSF. Moreover, since the axial PSF is a matter of interest in 3D imaging systems in which the lenses have high numerical aperture, such as confocal microscopy, we have extended the Toraldo method to the case of nonparaxially focused fields.

2. The axial response of apodized focusing systems

We start by considering the axial amplitude PSF of an aberration-free axially-symmetric focusing system in the nonparaxial scalar Debye approximation [18], that is

$$h(z) = \int_0^{\alpha} A(\theta)\exp\left(i2\pi \frac{n\cos\theta}{\lambda} z\right)\sin\theta \, d\theta \ , \tag{1}$$

where $A(\theta)$ represents the apodization function, i.e. the amplitude transmittance of the exit pupil, θ being the angular coordinate at the exit pupil plane as seeing from the focal point. z is the axial coordinate as measured from the focus of the system, α the semiaperture angle, λ the wavelength, and n the refractive index of the medium.

After performing the nonlinear mapping

$$\zeta = \frac{\cos\theta - \cos\alpha}{1-\cos\alpha} - 0.5 \ ; \quad q(\zeta) = A(\theta) \tag{2}$$

Eq. (1) can be rewritten as

$$h(z_N) = (1-\cos\alpha)\exp\left(i\pi\frac{1+\cos\alpha}{1-\cos\alpha} z_N\right)\int_{-0.5}^{0.5} q(\zeta)\exp(i2\pi\zeta z_N)\, d\zeta \ , \tag{3}$$

where we have expressed the axial position in the focal volume in terms of the normalized non-dimensional variable

$$z_N = \frac{n}{\lambda}(1-\cos\alpha)\,z \ . \tag{4}$$

From Eq. (3) it follows that the axial amplitude PSF is governed by the 1D Fourier transform of a nonlinearly mapped version of the pupil amplitude transmittance. Note that this formula is valid to describe the axial behavior of both paraxial (low aperture) and nonparaxial (high aperture) focusing systems. To obtain a desired axial distribution it is necessary to solve an inverse problem and design a mapped function $q(\zeta)$ such that its 1D Fourier transform approximates the desired form. The optical implementation of a filter whose transmittance maps to such $q(\zeta)$ is in general a non-trivial task. An alternative approach is based on the Toraldo concept. As we show below it is possible to design ring-shaped pupil filters to control the position of the zeros of the axial-amplitude PSF. In the shaping procedure the influence of the angular aperture is two-fold. On one hand, for a given function $q(\zeta)$ the actual form of the apodization function explicitly depends on the value of α through the mapping of Eq. (2). On the other hand, the axial extent of the focal spot is determined by the value of α through the scale factor of Eq. (4). This means that, in general, the optimal pupil transmittance for a given purpose does not scale linearly with α, and then must be calculated explicitly for the NA value of interest.

3. The Toraldo concept

Toraldo di Francia [12] showed that the radii of the zero-intensity rings in the focal plane of a paraxial focusing system can be selected at will by using a pupil filters subdivided into concentric zones with constant transmittance. A thorough description of Toraldo's procedure can be found elsewhere [13,16]. As remarked in Section 1, the aim of this work is to adapt the Toraldo concept to a new situation: the control of the shape of the axial PSF of nonparaxial focusing systems. We therefore outline the similarities and differences between the original Toraldo algorithm and our procedure. In the original algorithm the amplitude transmittance of the filter, $t(r)$, is subdivided into m concentric annular zones to control the radii of $m-1$ rings of zero intensity. In our approach the function $q(\zeta)$ is also subdivided into constant-transmittance zones. Here it is important to take into account that if one wants to tailor the axial PSF with the constraint that the transverse PSF should remain almost invariant, the resulting function $q(\zeta)$ must be centrosymmetric [3,4]. This implies that to control the positions of $m-1$ axial zeros, the interval [-0.5, 0.5] should be divided into $2m-1$ subintervals such that in each subinterval the function $q(\zeta)$ is constant. On the basis of the above reasoning, the procedure for shaping the axial intensity of nonparaxially focused scalar fields is as follows:

First, the function $q(\zeta)$ is decomposed as

$$q(\zeta) = \sum_{i=1}^{m} \left[k_i \ a_i(\zeta) - k_{i-1} \ a_{i-1}(\zeta)\right] \ , \text{ where } \ a_i(\zeta) = \begin{cases} \mathrm{rect}(\zeta / \Delta_i) & \textit{if} \quad i = 1,\ldots,m \\ 0 & \textit{if} \quad i = 0 \end{cases} \ . \tag{5}$$

In Eq. (5) $\Delta_m = 1$, $\Delta_i > \Delta_{i-1}$, $m \geq 2$, and k_i is the transmittance of the i^{th} zone.

According to Eq. (3) the axial amplitude PSF of this filter is given, apart from irrelevant constant factors, by the 1D Fourier transform of $q(\zeta)$, that is

$$h(z_N) = \sum_{i=1}^{m} k_i \ \tilde{a}_i(z_N) \ , \text{ where } \ \tilde{a}_i(z_N) = \Delta_i \ \mathrm{sinc}(\Delta_i \ z_N) - \Delta_{i-1} \ \mathrm{sinc}(\Delta_{i-1} \ z_N) \ . \tag{6}$$

In the simplest case, corresponding to $m = 2$, which allows one to control the position of one zero, the axial amplitude PSF is given by

$$h(z_N) = (k_1 - k_2)\Delta\,\mathrm{sinc}(\Delta\, z_N) + k_2\,\mathrm{sinc}(z_N) \ , \quad \text{with} \ \ \Delta = \Delta_1 \ . \tag{7}$$

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Normalizing to unity the axial PSF at its center, i.e. $h(0)=1$, we obtain

$$(k_1 - k_2)\Delta + k_2 = 1 \ . \tag{8a}$$

We now apply the design constraint, that is, we force the axial amplitude to be zero at a selected point, say $z_N = z_1$. Then from Eq. (7)

$$(k_1 - k_2)\Delta\,\mathrm{sinc}(\Delta z_1) + k_2\,\mathrm{sinc}(z_1) = 0 \ . \tag{8b}$$

Solving the set of Eqs. (8) yields

$$k_1 = \frac{\sin(\pi\Delta z_1) - \sin(\pi z_1)}{\sin(\pi\Delta z_1) - \Delta\sin(\pi z_1)} \quad \text{and} \quad k_2 = \frac{\sin(\pi\Delta z_1)}{\sin(\pi\Delta z_1) - \Delta\sin(\pi z_1)} \ . \tag{9}$$

Note from Eqs. (5) and (9) that the function $q(\zeta)$, and therefore the coefficients k_i, which produces an axial intensity that is symmetric around the focus, is real. If, additionally, one wants to maximize the filter throughput, the zones should have no absorption and have opposite phases, that is, $k_2 = -k_1$. The choice of a phase-only filter yields a design that can be manufactured with relative ease. From Eq. (9) we find

$$\Delta = \frac{\mathrm{sinc}(z_1)}{2\,\mathrm{sinc}(\Delta z_1)} \ . \tag{10}$$

Eq. (10) is a transcendental equation. The root of the equation is the abscissa of the point of intersection of the curve $y = \mathrm{sinc}(z_1)/\mathrm{sinc}(\Delta z_1)$ and the straight line $y = 2\Delta$.

To establish the usefulness of the method we consider the case of an axially superresolving pupil filter, that is, a filter that shapes the axial PSF such that the extent of the central lobe is narrowed by an arbitrary factor, which we set to 0.7 in the present example. Since in the case of the circular pupil, for which $q(\zeta) = \mathrm{rect}(\zeta)$, the first axial zero appears at $z_N = 1$, then the value of the constraint parameter should be $z_1 = 0.7$. The solution of the transcendental Eq. (10) is then $\Delta = 0.19$. In Fig. 1 we compare the normalized axial intensity PSF provided by the Toraldo-like filter with that corresponding to the nonapodized circular pupil. In addition, we have represented, in contour plots, the 3D intensity distribution in the meridian plane for both cases. These are of interest because they show that there are no significant off-axis lobes, which was not guaranteed by the design. Due to the centrosymmetry of the Toraldo filter the central lobe in the transverse direction remains almost invariant.

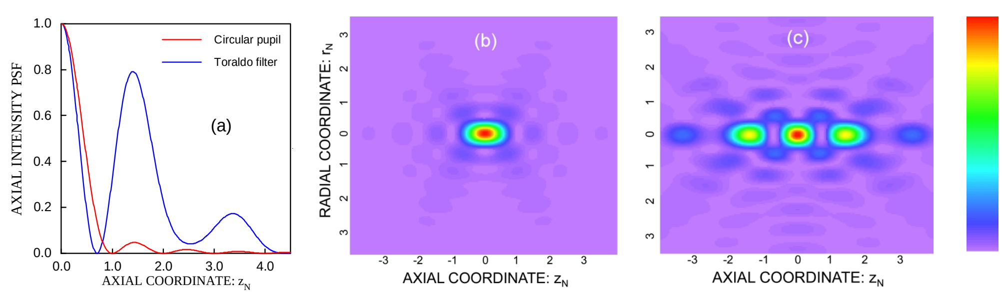

Fig. 1. (a) Normalized axial intensity PSF corresponding to the circular pupil and to the three-zone Toraldo filter; (b) contour plot of the 3D intensity PSF in the meridian plane corresponding to the circular pupil; (c) as b) but for the three-zone Toraldo filter. The normalized radial coordinate is $r_N = (n/\lambda)\,r\sin^2\alpha$.

Next, to show the influence of the semiaperture angle α into the actual form of the pupil filter, in Fig. 2 we plot the function $q(\zeta)$ together with the 2D representation of the actual amplitude transmittance of the filter for two different values of α. Note that a similar effect, but in a different context, was found in [5-9].

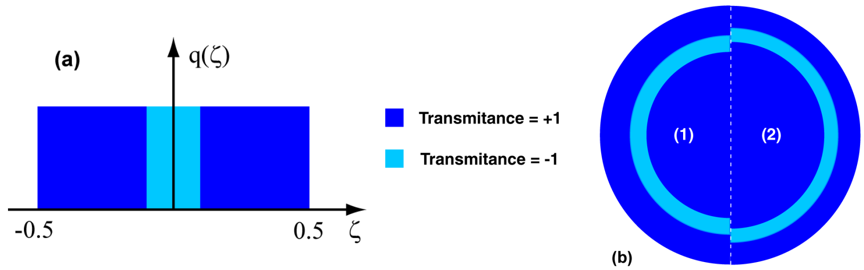

Fig. 2. Three-zones Toraldo filter: (a) mapped transmittance; (b) actual 2D representation for the cases of $\alpha = 10°$ (1) and $\alpha = 67.5°$ (2). Note that the form of the pupil filter strongly depends on the value of α .

4. Application to Confocal Microscopy

The use of the three-zone Toraldo filter gives rise to an axial distribution in which the narrowness of the central lobe is accompanied by the enlargement of outer lobes. When dealing with some 3D imaging systems in which the axial resolution is of great importance, e.g. confocal fluorescence microscopes, this problem can be overcome. This is because the intensity PSF of the confocal system results from the product of the PSFs of the illuminating and the collecting systems. In a confocal fluorescence system the illumination of the sample and the collection of light are two statistically-independent processes. If one modifies the transmittance of the pupil of one of the arms, the corresponding PSF is shaped while the PSF of the other arm remains unaffected.

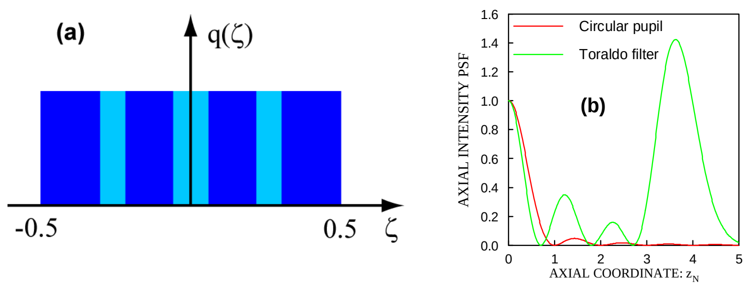

Fig. 3. (a) Seven-zone Toraldo filter designed for obtaining axial superresolution in confocal fluorescence microscopy. The filter was calculated for the case of $\alpha = 67.5°$; (b) axial intensity PSF.

What we propose here is to place the Toraldo filter in the illumination pupil and a circular aperture in the collection (to avoid light losses in the collection process). In this case, the resulting confocal-system PSF is narrowed in the axial direction, compared to the corresponding to the nonapodized confocal system, and the high axial sidelobes disappear. However, in fluorescence applications we should consider that the presence of high sidelobes in the illumination PSF could result in significant photo-bleaching of the 3D specimen at the positions of the strong secondary peaks. This problem can be overcome by designing filters with higher number of zones. Specifically we found that with a seven-zone Toraldo filter one can control the effect of photo-bleaching by sending the huge axial sidelobe further away. Note that in this case the design procedure is adapted to the thickness of the fluorescent sample, to ensure that the axial sidelobes remain outside the sample during imaging. In Fig. 3 we have plotted the mapped transmittance of the seven-zone filter, together with its normalized axial intensity PSF. In this case we see a significant reduction in the width of the central lobe, and samples up to $z_N = 4$ thick can be imaged without bleaching by the sidelobes. Of course, if one wants to send the

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huge axial sidelobe further away it is necessary to design a Toraldo filter composed by a higher number of zones. A practical rule for the design procedure is that the position of the huge sidelobe is approximately $z_N = m+1$.

Another application of Toraldo filters is in reflection bright-field confocal microscopy. Here also the PSF of the system results from the product between the PSFs of the illuminating and collecting processes. However, in this case the height of the axial sidelobes does not constitute a problem. The requirements for the design are: (a) to narrow the central lobe of the illumination axial PSF, in order to reduce the width of the central lobe of the confocal PSF; (b) to position the zeros of the illumination axial PSF so that they coincide with the maxima of the collection axial-PSF sidelobes. This minimizes the effect of the sidelobes in the confocal PSF, which was not the case in other approaches for obtaining axial superresolution [4,15]. Taking into account these requirements we designed the seven-zone pupil filter shown in Fig.4(a). In this figure we also show the contour plots of the 3D PSF for a bright-field confocal instrument with two circular pupils (Fig. 4(b)) and for the confocal system but with the Toraldo filter in the illumination set (Fig. 4(c)).

Note that the use of the seven-zone Toraldo filter narrows the intensity PSF, alog the optical axis, and therefore provides an important improvement of the optical sectioning capacity of the microscope.

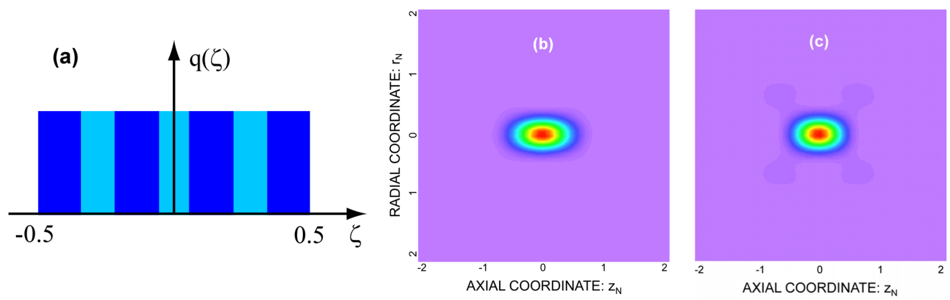

Fig. 4. (a) Seven-zone Toraldo filter designed to obtain axial superresolution in reflection confocal microscopy. The filter was calculated for the case of $\alpha = 67.5°$; (b) 3D intensity PSF of a reflection confocal microscope with two circular pupils; (c) as b) but with the seven-zone Toraldo filter in the illumination system.

5. Conclusions

The axial form of the Toraldo pupil synthesis method has been presented. The reported method allows the design of filters with the ability to control the positions of the zeros of the axial PSF of a lens, while preserving the structure of the transverse PSF. We show that, given a desired shape for the axial PSF, the actual form of the Toraldo filter explicitly depends on the aperture of the lens. The resulting filters, whose main feature is their simplicity, can be applied to improve the performance of 3D image formation systems, such as fluorescence and bright-field reflection confocal microscopes.

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