Reduction of focus size in tightly focused linearly polarized beams

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The electromagnetic theory predicts that when a linearly polarized collimated field is focused by a high-angle focusing system, components perpendicular to the initial polarization are generated. The use of annular masks to reduce the area of the focal spot usually increases the magnitude of this phenomenon, known as depolarization. We present a class of masks, the three-ring masks, which are important because they narrow the central lobe of the focal intensity distribution without increasing the depolarization. This can be very useful in modern optical applications, such as confocal microscopy or multiphoton scanning microscopy. © *2004 American Institute of Physics.*
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Improvement in the resolution of optical microscopes has been the aim of many research efforts during the last decades. In the search for optical setups that produce the maximum resolution, emphasis is generally placed on the use of modern microscope objectives with the highest available numerical aperture (NA). But even using the best optical elements, conventional wide-field microscopes cannot always meet the requirements of biological imaging. This is because when conventional microscopes are used to image three-dimensional (3D) samples, an image focused at a given depth also contains blurred information from the rest of the sample. To overcome this drawback, the use of new techniques such as confocal, multiphoton or multiple-axis microscopy have been proposed,[1,2] and to optimize the performance of such optical sectioning techniques the use of annular filters has been proposed.[3–5] However, as has been shown by several groups,[6–9] the insertion of annular apertures in high-angle focusing systems produces a significant increase of the relative strength of field components that are perpendicular to the polarization of the incident beam. This phenomenon, known as depolarization, can produce a sharper focus when the incident beam is radially polarized,[10] but produces significant broadening of the focal spot in the case of a linearly polarized incident beam. Contrary to the predictions of the scalar diffraction theory, annular filters often deteriorate the resolution of tightly focused systems using linearly polarized illumination. In this context, the aim of this letter is to show that this is not a general law, and that it is possible to design annular pupil filters that increase the resolution capacity of optical-sectioning microscopes, but prevent the detrimental increase in the strength of the depolarization effect.

To this end, we consider a monochromatic x-polarized plane wave front that impinges on an aberration-free high-aperture spherical lens (see Fig. 1). Assuming that the sine condition holds, and following the electromagnetic focusing theory of Richards and Wolf,[11] the electric-field distribution in the focal region is given by

$$\mathbf{E}(r,z,\varphi) = [I_0(r,z) + I_2(r,z)\cos 2\varphi]\mathbf{i} + I_2(r,z)\sin 2\varphi\mathbf{j} + i2I_1(r,z)\cos\varphi\mathbf{k}, \quad (1)$$

where $I_{0,1,2}$ are integrals over the aperture angle θ,

$$I_0(r,z) = \int_0^\alpha P(\theta)\sqrt{\cos\theta}(1+\cos\theta)J_0(kr\sin\theta) \times\exp(ikz\cos\theta)\sin\theta d\theta,$$

$$I_1(r,z) = \int_0^\alpha P(\theta)\sqrt{\cos\theta}\sin\theta J_1(kr\sin\theta) \times\exp(ikz\cos\theta)\sin\theta d\theta,$$

$$I_2(r,z) = \int_0^\alpha P(\theta)\sqrt{\cos\theta}(1-\cos\theta)J_2(kr\sin\theta) \times\exp(ikz\cos\theta)\sin\theta d\theta. \quad (2)$$

In Eq. (1), $P(\theta)$ accounts for the amplitude transmittance at the pupil of the lens and α is the maximum value of the aperture angle θ. The parameter φ represents the angle between the polarization direction of the incident field (assumed without any loss of generality to be in the x direction) and the meridian plane under study. In the case of weak focusing, i.e., for low values of α, functions I_1 and I_2 are very small as compared with I_0. Therefore, the focused field is basically x polarized and given by $I_0(r,z)$. In this case (the so-called paraxial case), the near and far fields are related by a Fourier transformation (note that for small α, $\cos\theta \approx 1$),

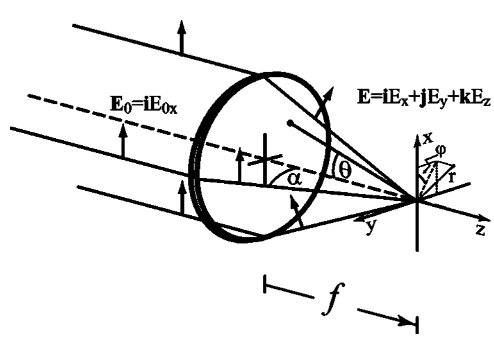

FIG. 1. Raytracing model for the focusing of an x-polarized incident electric field. Note that the depolarization is stronger for rays impinging the outer part of the lens aperture.

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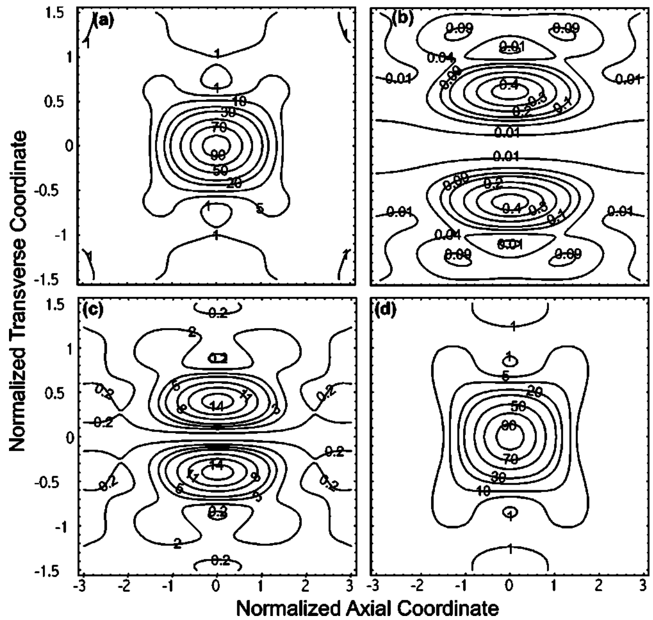

FIG. 2. Contour maps, for an objective with NA=1.2 (water) and a clear circular aperture, of the following normalized intensities: (a) $|E_x(r,z,\varphi=0)|^2$; (b) $|E_y(r,z,\varphi=\pi/4)|^2$; (c) $|E_z(r,z,\varphi=0)|^2$; and (d) $|\mathbf{E}|^2=|E_x|^2+|E_y|^2+|E_z|^2$ in plane $\varphi=0$. Axial and transverse coordinates are expressed in units of λ. The normalization in all the plots is such that $|\mathbf{E}(r=0,z=0)|^2=100$.

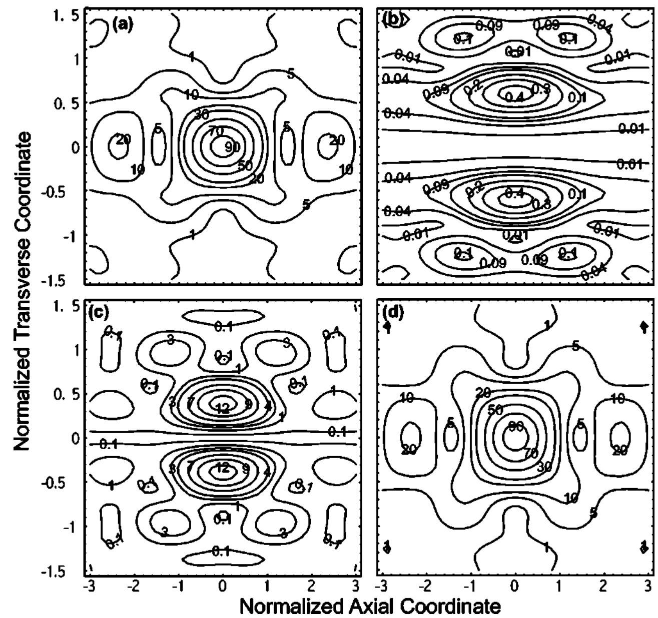

FIG. 3. Same contour maps as in Fig. 2, but for the 3R filter.

and the focused field can be accurately calculated using the scalar diffraction theory.[12] However, for higher values of α, the relative strengths of I_1 and I_2 increase so that significant components perpendicular to the initial polarization appear. As is clear from Fig. 1, this depolarization is more significant for rays impinging on the outermost part of the aperture, because these rays are more strongly inclined when focused.

Note from Eq. (1) that, due to the factors depending on φ, the field components are not radially symmetric. Specifically, the y-polarized focal field is null at the meridian planes (x,z) and (y,z), and is divided into four axially elongated lobes. It shows a structure similar to a well-known transverse electromagnetic (TEM) spherical-resonator mode; the TEM_{11}. The z-polarized focal field is null at the meridian plane (y,z) and is divided into two lobes like a TEM_{10} mode. To illustrate the importance of the depolarization phenomenon in strongly focused beams, in Fig. 2 we depicted the total normalized focal intensity and its components in the x, y, and z directions. For the calculation, we considered a clear circular aperture for the focusing lens and NA=1.2 (in water). For each polarization component, we have selected the meridian plane in which its strength is maximal. Note that the intensity peak of the y- and z-polarized focal fields, respectively, amount to 0.46% and to 15.20% of the x-polarized component.

Strong focusing is the key to obtain high resolution in optical systems. Among such systems, confocal scanning microscopes constitute one of the most widely used techniques in the area of 3D imaging. To optimize the lateral resolution of such systems, the use of annular masks has been proposed. However, as shown by Gu and co-workers,[7] depending on the type of incident polarization the use of annular masks can produce a splitting effect in the focal spot. In other words, the relative strengths of field components perpendicular to the initial polarization (mainly the z component) significantly increase, giving rise to a focus that is split into two peaks. When this occurs, the expected lateral super-resolution is strongly deteriorated. Besides this effect, the annular masks generate a 3D focal distribution that varies very slowly along the propagation direction, resulting, in the limit case, in a zero-order Bessel beam.[13]

Here, we propose the use of a special class of annular mask, the so-called three-ring (3R) filters, to improve the optical sectioning capacity of the above-mentioned imaging systems. The filters have the ability to reduce the size of the focal intensity distribution in the axial direction, but avoid the increase of depolarization. The masks consist of the concentric superposition of two opaque rings centered on a circular pupil. These filters produce an effect similar to the Young's double-slit experiment, but oriented along the optical axis. In terms of this experiment, it is easy to explain the axial behavior of the filters. The inner and outer rings produce an axial pattern in which the central lobe is narrowed but the sidelobes are high (as in a Young's experiment where the slits have a finite width). The central ring mainly contributes to the height of the central lobe. The combination of both of these effects results in an axial pattern with a narrow main peak and low sidelobes.[14] The filters have the following features: (a) They can narrow, between certain limits, the central lobe of the axial amplitude distribution; (b) they limit the increase of the axial sidelobes (otherwise inherent to the use of annular filters); (c) the lateral extent of the focal spot remains almost unaltered; and (d) they prevent an increase of the depolarization.

The latter feature is the key aspect that we address in this letter. It occurs because the enhancement of depolarization due to the outer-most annulus is compensated for by the part of the wave front that impinges on the central zone of the mask.

To demonstrate the utility of the filters, in Fig. 3 we have repeated the same calculations shown in Fig. 2, but the clear circular aperture is substituted by a 3R mask. The normalized radii of the rings were: $r_1=0.61$, $r_2=0.73$, $r_3=0.81$, $r_4=0.89$, and $r_5=1.0$. The light throughput of the mask is 70.3%. Note from the figure that the use of the 3R filter produces a 14% reduction in the axial width of the central

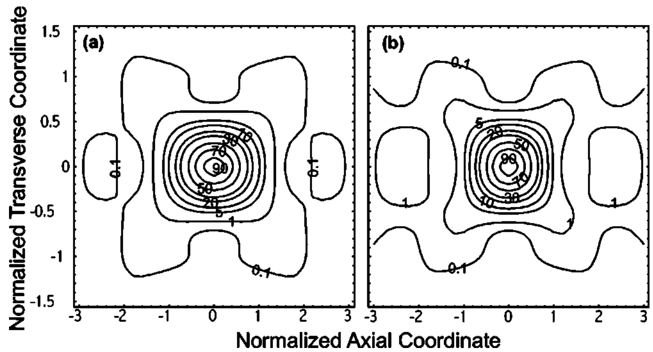

FIG. 4. Contour maps of the two-photon fluorescence intensity, $|\mathbf{E}|^4$, in the meridian plane $\varphi=0$ for the cases: (a) a clear circular aperture; and (b) the 3R mask.

lobe of the 3D focal intensity distribution. The intensity peaks of the *y*- and *z*-polarized components are 0.43% and 13.24% of the *x*-polarized component. Note that the use of the proposed 3R filter slightly decreases the magnitude of the depolarization. 3R filters can therefore be used effectively to improve the axial resolution of confocal scanning microscopes.

The utility of 3R filters is more appreciable in case of two-photon excitation (TPE) scanning microscopy, which is a nonlinear technique that relies on the simultaneous absorption of two photons, following which a single fluorescence photon is emitted.[15] The excitation wavelength is typically twice that used in the single-photon case. TPE scanning microscopes inherently possess optical sectioning capacity. This is because the fluorescence intensity is proportional to the square of the illumination intensity. Such a squared relation allows a significant reduction of the magnitude of the depolarization effect. Note that now the fluorescence intensity peak corresponding to the *y*- and *z*-polarized fields are reduced to 0.002% and to 1.7% of the *x* component, respectively. In Fig. 4, we have depicted the numerically evaluated two-photon fluorescence intensity distribution in the meridian plane $\varphi=0$ for a NA=1.2 (water) objective with circular aperture [Fig. 4(a)] and with the 3R mask [Fig. 4(b)]. These calculations demonstrate that the 3R filter narrows the axial extent of the focal intensity distribution by 13%. As a measure of the enhancement obtained with our method, we note that the same width would be obtained by using an objective with a clear circular aperture and NA=1.24.

In summary, we have presented a focus engineering technique that allows a significant reduction of the axial extent of the 3D focal intensity distribution while preventing the undesirable increase of the depolarization effect inherent in high-aperture focused linearly polarized beams. The technique can be very useful to improve the 3D resolution capacity of optical sectioning fluoresce imaging techniques like single-photon confocal scanning microscopy and TPE scanning microscopy.

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