Creating $\lambda/3$ focal holes with a Mach–Zehnder interferometer

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ABSTRACT We report the formation of doughnut-shaped focal intensity distributions with hole diameters of $\lambda/3.3 \approx 232$ nm full-width-at-half-maximum. The doughnut shape is created by illuminating a high-numerical-aperture lens with the output of a Mach–Zehnder interferometer, in which half of the wavefront in each arm is phase retarded by $\pi$. The focal intensities are probed with a point-like scatterer and compared with the predictions of a vectorial focusing theory. The orientation of the phase-discontinuity line with respect to the electric field determines whether a strong longitudinal or a vanishing electric field is produced at the focal point. Conditions are given for creating high-contrast focal holes at the sub-micron scale.

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1 Introduction

In recent years focal intensity distributions of high-numerical-aperture (NA) lenses other than the ordinary intensity point-spread function (I-PSF) have attracted considerable attention. This applies in particular to doughnut-shaped I-PSFs featuring in the center either no intensity or fields oriented along the optic axis. The latter facilitate the observation of transition dipole moments in single molecule experiments [1–4] and can be used to enhance light fields at sharp metal tips [5]. I-PSFs with vanishing central intensity play a crucial role in the concept of stimulated emission depletion (STED) fluorescence microscopy [6, 7]. In this concept, a doughnut-shaped beam depletes the excited molecules at the outer part of a regular focal spot by stimulated emission. Another application for doughnut-shaped focal modes is the focusing of atoms [8–11]. Blue detuned laser beams can be used to direct atoms towards the low intensity in the center. Compared with red detuned ordinary foci, in which the atoms accumulate at high intensities, absorption is avoided, and therefore so is noise induced by spontaneous emission as well as Stark shifts.

The theory of doughnut-shaped intensity distributions is related to that of laser cavity modes, which have been extensively examined [12]. However, the conditions studied herein are notably different. Our doughnuts need not obey resonator boundary conditions. Besides, the doughnuts should be as small as possible, with holes on the sub-micron scale. For this reason lenses of high numerical aperture (NA) are required, which means that in the creation of the doughnuts the vectorial properties of light will play a role. Doughnut-shaped I-PSFs based on purely radially or tangentially polarized light beams passing through high-NA lenses have been numerically simulated, albeit only recently [13]. For the implementation of these modes in microscopy, either active or passive elements can be used in the path of the incident light. Active elements, like flexible mirrors [14] or ferroelectric liquid-crystal spatial light modulators, [15] provide the opportunity of feedback control. However, they are more expensive and more difficult to implement than passive elements, such as mode transformers using astigmatic lens systems [16–18], space-variant dielectric sub-wavelength gratings [19], and polarization controlled fibers [20], or by using phase shifting of part of the expanded laser wave fronts in a Mach–Zehnder interferometric setup [13, 21–23]. The latter has proved robust in operation and has recently been used in applications such as measurements of the longitudinal transition dipole moments of single molecules [3] or stimulated emission depletion (STED) fluorescence microscopy [7].

In this paper, we report on a theoretical and experimental study on producing sub-micron-scale focal doughnuts with a Mach–Zehnder interferometer and investigate their practical implementation. The use of a Mach–Zehnder interferometer to create radially or tangentially polarized light has been suggested by Youngworth et al. [23]. We note that this interferometer does not produce a purely radial or tangential polarization, since the polarization distribution over the rear aperture of the objective lens is approximated by four quadrants, each containing a linear polarization of $\pm45^\circ$ with respect to the $x$ axis, such that the polarization of neighboring quadrants is perpendicular. In the following we consider this polarization distribution in our calculations and, additionally, we expand this study to finite-sized beams by taking into account the diameter of the pinholes providing the illumination...
wavefronts. Moreover, by realizing the concept and by measuring the doughnuts, we compare experimental data with theory. Since first applications of z-polarized E-field components have been reported [1–4, 24, 25], we shall confine ourselves to the creation of vanishing central intensities. An important application of such doughnuts is STED-microscopy [7]. In this ultrahigh-resolution fluorescence microscopy technique, a second doughnut-shaped pulsed intensity distribution is created around the focal area after a first laser pulse of shorter wavelength has excited the fluorescent dyes in the focal volume. The second pulse induces stimulated emission and therefore switches the excited molecules off at the rim of the focal area, hence decreasing the fluorescing volume.

2 Setup

The doughnut-shaped focal intensity distribution was created by a Mach–Zehnder type setup [12, 21, 23] as sketched in Fig. 1. The polarization of a laser beam from a mode-locked Ti:Sapphire laser (Coherent Inc., 76 MHz, 120 fs) operating at a wavelength of 765 nm was rotated by 45° using a λ/2 plate. A polarizing beam splitter PBS1 divided the beam into two orthogonally polarized beams. Rotation of the λ/2 plate allowed for exact balancing of the light intensity in the two arms of the interferometer. Both beams were expanded and cleaned by focusing on pinholes of variable size. After expansion, the beam encountered glass plates of λ/10 flatness, covered on one half with a 1 μm thick layer of MgF2 so that a phase retardation of π was introduced. We now define the orientation of these phase-plates by the dividing line between the two halves. In plate PU the dividing line is oriented parallel to the setup plane, that is x-oriented, whereas in PR the dividing line is vertical or y-oriented.

Two different arrangements of the phase-plates with respect to the E-field were examined. First, the dividing line of the phase plates was oriented parallel to the transmitted E-field, as shown in Fig. 1. That is, PU was inserted into the upper, x-polarized beam. Similarly, PR was inserted into the y-polarized arm. In the second study, the orientation of the dividing lines was reversed, so that the phase-plates were perpendicular to the E-fields of the impinging wavefronts. The upper arm of the interferometer could be adjusted in length using a piezo-mounted mirror. After their recombination at the polarizing beamsplitter PBS2, the combined beams were directed, via a flip-mirror FM, towards a dichroic mirror (DC) that reflected approximately 99.3% of the light into the objective lens (Leica Plan Apo, 100×, Oil, 1.4 NA). The lens also collected a fraction of the back-scattered light from a tiny gold bead that was employed for probing the I-PSF. Of this light 0.7% was transmitted by the dichroic mirror and detected by a photomultiplier tube (PMT). The dichroic mirror was oriented with a steep angle to the beam to minimize the difference in transmittance for s- and p-polarized light. The 300 μm opening of the PMT housing and the 120 mm focusing lens did not act as confocal spatial filters. Switching off the flip-mirror FM opened the path for combining the parallel wavefronts on a CCD camera. A 45° oriented polarizer placed in front of the camera allowed the initially orthogonal beams to partially interfere and reveal their phase difference δ on the camera.

3 Numerical results

The I-PSF was computed by numerically evaluating the Debye integral [26] for each component of the converging light field. For linear polarization of the initial field, with a polarization angle φ0 to the x axis, the focal field E(r) of an aplanatic lens is given by

$$E(r) = \int_0^\alpha \int_0^{2\pi} d\phi E_0(\theta) \sqrt{\cos \theta} \sin \theta e^{i(\Psi(\theta, \phi) + k(s-f))} \left(\cos^2(\phi - \phi_0) \cos \theta + \sin^2(\phi - \phi_0) \sin(\phi - \phi_0) \cos(\phi - \phi_0) \cos \theta - 1\right).$$

where φ and θ are the azimuthal and polar angles, respectively, and α denotes the angle of the half-aperture, with 0 ≤ θ ≤ α. We assumed α = 64.2°, because it matched best the nominal value of NA = 1.4 engraved on our lens. c is the speed of light, and f the focal length. s is the path traveled by the light from the point on the converging spherical wave front with the coordinates (f, θ, φ) to the position r in the focal region. The focal point is located at r = (0, 0, 0). λ is the vacuum wavelength and k = 2π n/λ the wave number, with n being the refractive index. To match experimental conditions, a wavelength of 765 nm and a refractive index of 1.518 were used in the calculations.

The phase function $\Psi(\theta, \phi)$ at the exit pupil of the lens describes the phase deviation from a reference sphere. In (1) the amplitude of the incident field $E_0(\theta)$ is still assumed to be constant over the entrance pupil. $\Psi(\theta, \phi)$ was eventually modified through the orientation of the phase plates in the Mach–Zehnder interferometer.

The expression in (1) allowed us to calculate the focal field distribution $E_1$ resulting from one arm of the interferometer.
The second arm, featuring a polarization angle \( \varphi_0 + 90^\circ \), rendered \( E_2 \). Considering the phase shift \( \delta \) between the arms we express the focal electric field as

\[
E = E_1 + e^{i\delta}E_2 = \begin{pmatrix}
E_{1,x} \\
E_{1,y} \\
E_{1,z}
\end{pmatrix} + e^{i\delta} \begin{pmatrix}
E_{2,x} \\
E_{2,y} \\
E_{2,z}
\end{pmatrix}.
\]

The resulting focal intensity distribution is

\[
I = I_x^2 + I_y^2 + I_z^2 \propto |E_{1,x} + E_{2,x}e^{i\delta}|^2 + |E_{1,y} + E_{2,y}e^{i\delta}|^2 + |E_{1,z} + E_{2,z}e^{i\delta}|^2.
\]

The effect of the phase retarding plates PU and PR is described by

\[
\Psi_{\text{ PU}}(\theta, \varphi) = \begin{cases}
\pi & \text{for } 0 \leq \varphi < \pi \\
0 & \text{otherwise}
\end{cases}
\]

\[
\Psi_{\text{ PR}}(\theta, \varphi) = \begin{cases}
\pi & \text{for } -\pi/2 \leq \varphi < \pi/2 \\
0 & \text{otherwise}
\end{cases}
\]

respectively. Figure 2 shows intensities for selected configurations. In the left column, Fig. 2a–c, the dividing line of the phase-retarding plates was oriented parallel to the respective fields and the respective relative phase \( \delta \) was 0, \( \pi/2 \), and \( \pi \). Each quadruple of Fig. 2 shows the modulus-squared of the three \( E \)-field components and their sum, i.e. the I-PSF. The grayscale in each single image is adjusted to fit the maximum, while the normalization within each quadruple is with respect to the maximum of the I-PSF. This quantifies the contribution of each polarization component. The size of each panel is 1.8 \( \mu \text{m} \times 1.8 \mu \text{m} \). Note that in Fig. 2a–c the intensity at the focal point vanishes.

Figure 2d–f shows the corresponding intensities for the phase plates oriented perpendicular to the field polarization. The relative phase \( \delta \) was again chosen to be 0, \( \pi/2 \) and \( \pi \), respectively. Figure 2d represents the interesting case of a \( z \)-polarized component dominating the center of the point-spread function. In fact, the field at the focal point is exclusively \( z \)-polarized, which is of great interest to microscopy [1–4, 25]. The panel of Fig. 2f exhibits vanishing intensity at the center. However, the drawback of this implementation of a central zero is that the relative phase \( \delta \) must be stabilized very accurately. This renders the setup unsuitable for a resolution increase by STED since any non-vanishing central field is counterproductive [27]. Most applications relying on a \( z \)-polarized intensity distribution (see Fig. 2d) are less sensitive to phase instabilities, because the fluctuations may be kept negligible.

The finite pinhole size is readily accounted for by a convolution of the above \( E \)-field with the demagnified geometric image of the pinhole in the focal plane of the lens. This is equivalent to adding the field contributions from across the whole pinhole area in the focal region. The results for 0, 20, and 40 \( \mu \text{m} \) diameter pinholes are displayed in Fig. 3. The phase-plate orientation was chosen to be parallel to the \( E \)-field orientation. The results show that the zero intensity in the center of the I-PSF is maintained regardless of pinhole size. This can be understood on the basis of symmetry considerations. Since the pinhole is centered on the optical axis, every point in the demagnified pinhole area has a point-symmetric counterpart featuring an \( E \)-field of the same amplitude and opposite phase. As a result the sum vanishes at \( r = (0, 0, 0) \). Another interesting outcome is the change of the doughnut fine structure with increasing pinhole size. Especially in the case of a 0° relative phase difference, there is a change from a more quadratic appearance to a ring-shaped one. An intuitive explanation for this result is that the increase of the pinhole size and the concomitant decrease of the beam diameter reduce the amplitude of the high spatial frequencies that pass through the lens. So in essence, the I-PSF is spatially low-pass filtered and hence smoother.
Depending on the relative phase projecting the recombined interfering beams onto a CCD camera beam. We controlled the relative phase of the two arms by parallel offset introduced by that movement could be neglected second to fourth rows, the relative phase \( \delta \) is 0, 90, and 180\(^\circ\), respectively. In the first row indicates the pinhole size used in the corresponding column. In the second to fourth rows, the relative phase \( \delta \) is 0, 90, and 180\(^\circ\), respectively. The I-PSF was probed by scanning of a 100 nm diameter gold bead through the focal volume. It has been reported that beads of this diameter can be regarded as point-like scatterers [28, 29]. The gold beads were spread onto a cover slip from a dilute solution and covered with immersion oil to ensure a constant refractive index surrounding. Using a three-axis piezo-stage (Melles Griot) with positioning feedback control, the positioning accuracy was 5 nm. A piezo-movable mirror (Fig. 1) was used to adjust the relative phase \( \delta \). Any parallel offset introduced by that movement could be neglected because of the comparatively large diameter of the expanded beam. We controlled the relative phase of the two arms by projecting the recombined interfering beams onto a CCD camera using the flip-mirror FM. The images are shown in Fig. 4. Depending on the relative phase \( \delta \), the quadrants in the recombined beam appear bright and dark, equally gray, or dark and bright for \( \delta = 0^\circ, 90^\circ, \) and \( 180^\circ \), respectively. In fact, the CCD camera provided an easy means of finding coherent overlap between the two arms of the interferometer, despite the fact that the coherence length of the femtosecond laser pulses was \( \sim 45 \mu m \) only.

Figure 5a–d shows the cross-section of the I-PSF in the focal plane, as rendered by the gold bead. In Fig. 5a and c the phase difference \( \delta \) was set to 0\(^\circ\), while in Fig. 5b and d \( \delta \) was 180\(^\circ\). In Fig. 5a and b 15 \( \mu m \) diameter pinholes were used, whereas in Fig. 5c and d the pinhole diameter was 30 \( \mu m \). The motivation was the following. For STED microscopy it is essential to optimize both for a deep hole and high power. Deep holes demand spatially cleaned beams and small pinholes, but high power throughputs require just the opposite. Although the finite pinhole size should have no influence on the intensity at \( r = (0, 0, 0) \), the experimental data of Fig. 5 reveal a degradation of the I-PSF with increasing pinhole size. Figure 5e illustrates an experimentally obtained focal doughnut as a two-dimensional surface plot. This doughnut was produced with an illumination pinhole diameter of 20 \( \mu m \). Due to aberrations the intensity maximum in the ring is slightly asymmetric. Normalized to the average intensity in the ring, the doughnut features a contrast ratio of \( 1 : 70 \) and a full-width-at-half-maximum of 236 nm.

As a figure of merit we quantified the intensity contrast by comparing the intensity at \( r = (0, 0, 0) \) with the average intensity in the ring. The line profiles through \( r = (0, 0, 0) \) are shown in Fig. 6 for pinhole sizes of 10, 15, 20, and 30 \( \mu m \) and \( \delta = 0^\circ, 90^\circ, \) and 180\(^\circ\) in Fig. 6a and b, respectively. For all measurements the dividing line of the phase plates was parallel to the \( \mathbf{E} \)-field orientation. In the experimental doughnuts of Fig. 5a–d the holes featured full-widths-at-half-maximum (FWHM) of 234, 230, 232, and 232 nm, respectively. In the same order, these values correspond to \( \lambda/3.27, \lambda/3.33, \lambda/3.30, \) and \( \lambda/3.30 \). For comparison, the hole FWHM of the calculated intensity distributions displayed in Fig. 2a and c measure 220 and 218 nm, respectively.

Contrary to the numerical results, which predicted a vanishing intensity at the focal point, the central depression of the PSF was degraded with increasing pinhole size, as shown in the measurements of Fig. 6. However, up to a pinhole size of 20 \( \mu m \) this degradation was marginal. In the case of \( \delta = 0^\circ \) in Fig. 6a, the local minima were 1.1, 1.2, 1.4, and 3.1\%, for 10, 15, 20, and 30 \( \mu m \) pinholes, respectively; for \( \delta = 180^\circ \) we found 1.3, 1.5, 1.7, and 5.3\%. Hence, a pinhole of up to 20 \( \mu m \) can be regarded as a point-like illumination source in our setup. Taking into account the wavelength of the laser,
FIGURE 5  a–d Experimental I-PSF in the focal plane, recorded for pinhole diameters of 15 and 30 µm and for parallel and perpendicular orientations of the dividing lines of the phase plates with respect to their impinging fields. Panels a and c are the experimental counterparts of Fig. 2a, that is, they were calculated for infinitely small pinholes. Similarly, panels b and d correspond to Fig. 2c. The panel sizes are 1.8 µm x 1.8 µm. e Surface plot of a doughnut with phase plates oriented parallel to the E-fields and a relative phase delay of 0° between the two arms. The pinhole size was 20 µm. The contrast between the low and the average intensity of the ring was 1:70. The full-width-at-half-maximum of the hole was 236 nm, corresponding to λ/3.3

FIGURE 6  Measured intensity profiles through the center of the doughnuts for various pinholes (10, 15, 20, and 30 µm). a δ = 0°, corresponding to the doughnuts shown in Fig. 5a and c, b δ = 180°, corresponding to the doughnuts shown in Fig. 5b and d. The data show that under the present aberration conditions and using a 100 x 1.4 NA magnification lens, pinhole diameters < 20 µm may be regarded as point-like sources.

ever, the agreement of Fig. 5a, recorded at δ = 0°, with Fig. 2a is inferior, because it is fairly ring-shaped. While the consideration of a finite-sized pinhole predicts a change in doughnut shape from a more cornered appearance to a ring-shaped one (Fig. 3), this change should be effective only for pinhole diameters larger than 15 µm. The slight but significant discrepancy in shape clearly hints at aberrations of the focused wavefront.

Next we provide further evidence against omissions in the phase difference measurements. By displaying the calculated and the experimental data on a logarithmic scale we scrutinize the structure of the side maxima in great detail. Figure 7a–c shows the calculated total intensities, whereas Fig. 8a and b are the experimental counterparts of Fig. 7a and c. The profiles of the side maxima are taken along the offset dashed lines. For δ = 90° and 180° the profiles of the calculated I-PSF show a minimum in the center, as indicated by an arrow. By contrast, in the case of δ = 0° there is a local maximum. Comparison of these findings with the corresponding profiles of the measured I-PSF of Fig. 8 support δ = 0° and 180° in the respective experiments. δ = 90° is improbable because the experimental profile data in Fig. 8a exhibit a central local maximum.

According to the experimental data of Fig. 6, for small pinholes the depth of the doughnut does not depend on whether δ = 0° or δ = 180°. This observation is not surprising as theory predicts zero intensity for all δ. Therefore, provided that the dividing lines of the phase plates are parallel to the individual E-fields, a tight control over the relative phase is irrelevant (see Fig. 2a–c). This is not so with the reversed phase-plate orientation used for generating z-polarized light, as calculated in Fig. 2d–f. Here, strict phase control is mandatory for creating either a strong z-polarized intensity (0°) or a hole (180°). In the first case, the finite pinhole size does not challenge the prominence of the z-polarized component in the center of the PSF (data not shown). Moreover, the calculations yield that the x- and y-oriented field components vanish at r = (0, 0, 0), regardless of the pinhole size. Still,
due to aberrations, small transverse intensity components of similar strengths as the minima of Fig. 6 can be anticipated in practice.

By a change in optical path length corresponding to the coherence length of the laser, the Mach–Zehnder interferometer may be detuned to incoherent superposition. In our case this amounted to \( \sim 45 \, \mu m \). The expression in (3) is then rewritten as

\[
I = I_x^2 + I_y^2 + I_z^2 = |E_{1,x}|^2 + |E_{2,x}|^2 + |E_{1,y}|^2 + |E_{2,y}|^2 + |E_{1,z}|^2 + |E_{2,z}|^2.
\]  

(6)

The numerical results are shown in Fig. 9a and b for \( E \)-fields oriented parallel and perpendicular to the dividing lines, respectively. Whereas in (a) the central intensity vanishes, a substantial contribution of \( z \)-polarized light appears in (b). This is in accordance with previously published measurements [7] of incoherently superposed \( E \)-field beams with parallel orientation. Again, the color maps are normalized to the maximum of the total intensity.

To compare the resulting brightness of the coherent with the incoherent case (Fig. 2 with Fig. 8), the absolute peak values are given in Table 1. With this table, not only are the total intensity images in Figs. 2 and 9 accessible, but so are the peak intensities of each polarization. For example, the \( z \)-component in Fig. 9b is \( 0.81 \times 3.35 = 2.71 \), which is exactly half of the \( z \)-polarized intensity of its coherent counterpart of Fig. 2d with \( \delta = 0^\circ \). The latter is equivalent to perfect constructive interference of the longitudinally po-

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**TABLE 1** Maxima (in arbitrary units) of the total intensities of Figs. 2 and 9. \( \delta \) denotes the relative phase of the \( E \)-fields of the interferometer arms.
lized fields from each of the arms. The factor of two is expected because for \( \delta = 0^\circ \) the coherent sum yields \( (E + E)^2 = 2(E^2 + E^2) \). This finding is relevant to applications in which \( z \)-polarized light is used. A tight control of the phase of the two beams gains a factor of two in \( z \)-polarized intensity.

5 Summary

We have presented a comparative theoretical and experimental study of the generation of doughnut-shaped focal intensity distributions at the sub-micron scale, using a Mach–Zehnder interferometer. In this setup two plane waves with a phase retardation of \( \pi \) over one half of the wavefront are coherently superposed before entering the back aperture of an objective lens. The orientation of the \( E \)-field with respect to the dividing line between the retardation zones leads to distinct cases. Perpendicular orientation creates strong longitudinal fields in the focus, but the relative phase of the two arms of the interferometer has to be strictly controlled. Parallel orientation of the \( E \)-field with respect to the dividing line is relevant to stimulated emission depletion microscopy. In theory the center of the I-PSF vanishes regardless of the pinhole size or relative phase. As phase control can be neglected, this is a particularly promising approach to producing diffraction-limited focal doughnuts at the sub-micron scale. If the interferometer is detuned such that there is no coherent overlap, the intensity of the \( z \)-polarized component is decreased by a factor of two. In some applications this may be beneficial for reducing experimental constraints.

In conclusion, our comparative study shows that sub-micron-scale doughnuts can be produced. The limiting parameter for generating the highest possible contrast ratio is wavefront aberration. Meticulous counterbalancing of aberrations should lead to sub-micron-scale doughnuts with contrasts > 1 : 500 in the future.

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REFERENCES