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Compromising resolution and contrast in confocal microscopy using a novel obstruction of circular pupils

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We study theoretically an amplitude pupil obstructed by a simple method permitting to improve the microscope resolution with a little degradation of the image contrast. Using confocal scanning optical microscope the resultant point spread function was calculated, from which the image of a point object is deduced. The calculations are made for two different pupils and compared with full circular aperture.

1. Introduction

The coherent microscope [1] offers an important advantage of apodization if compared to conventional optical ones (by apodization we mean the attenuation of the light energy in the outer rings). This property much improves the microscope resolution and is due to the fact that the resultant point spread function (PSF), when using confocal microscope, is sharpened [2]. In this case the resultant PSF depends on the characteristics of both the objective and the collector lenses, whereas the ordinary PSF for the conventional microscopes depends mainly on the objective lens.

Annular apertures used in confocal scanning optical microscope (CSOM) give theoretically a better resolution but with a decrease in contrast from the objects extended. This degradation in contrast occurs usually when the aperture is obstructed, e.g., annular one, hence, a smaller part of energy appears in the central disc its relatively greater part being thrown into the outer rings.

Certainly, the wavelength plays an important role in the microscope resolution, the resolution of an image obtained using UV-rays is, for example, better than that obtained with red light. In our analysis, however, we suppose monochromatic light for the microscope illumination.

In the recent work [3] we have shown theoretically that by using amplitude pupils of linear and quadratic distribution the CSOM resolution can be improved with respect to full circular pupil calculations. We have also studied the effect of spherical aberation on the microscope resolution using apertures of amplitude and phase modulation [4].

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Since in our opinion the above pupils are of purely mathematical nature, their construction requires, e.g., computer-generated filter [5] or thin film techniques, we have suggested simple apertures consisting of circles and annuli arranged by the method that permits the improvement of the microscope resolution with a simultaneous little degradation of the image contrast. The suggested arrangement of pupils was selected having in mind the attenuation of the low spatial frequency of the aperture, and the enhancement of the high spatial components. The insertion of the circles around the external annulus from the inside gave a quite good contrast compared to annular apertures particularly during the imaging of extended objects. Theoretical analysis with results and discussion will be given in the following Sections.

2. Calculation of the point spread function using CSOM

We shall calculate the point spread function for a pupil consisting of an annulus and four equal apertures arranged symmetrically around the corners (Fig. 1).

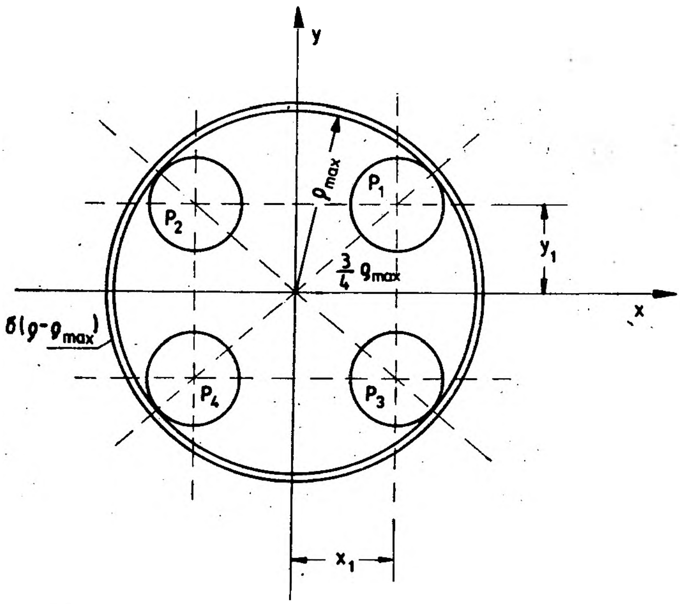

Fig. 1. Pupil consisting of an external annulus and four equal circular apertures arranged at the corners

The radius and the location of each of these circles are given from the boundary conditions. The proposed pupil is considered to have four-fold symmetry and it can be described by the following formulae:

$$P(\rho) = \delta(\rho-\rho_{\max})+\sum_{i=1}^{4} P_i(\rho) \tag{1}$$

where $\delta(\rho-\rho_{\max})$ is the Dirac function representing the annulus at the position $\varrho = \varrho_{\max}$, and

$$P_i(\rho) = \begin{cases} 1, & \text{if } |\rho-\rho_i| \leqslant \varrho_{\max}/4, \\ 0, & \text{otherwise.} \end{cases} \tag{2}$$

It is to be noted that each of the four circles has a radius $\rho_{max}/4$ and is located at a distance $\rho_i = (3/4)\rho_{max}$.

Now two-dimensional Fourier transformation is applied to Eq. (1) to obtain the expression for the point spread function, i.e.:

$$\mathrm{PSF}(W) = aJ_0(W)+\beta[J_1(W_1)/W_1]\sum_i \exp[-jk\rho_i r] \tag{3}$$

where $W = k\varrho_{max} r/f$, $W_1 = W/4$, $k = 2\pi/\lambda$ is the wave number and a and β are positive constants depending on the optical and geometrical properties of the pupil function:

$$\varrho_i^2 = x_i^2+y_i^2,\ r^2 = x'^2+y'^2,\ x_i = y_i = (3/4\sqrt{2})\varrho_{max},\ a = 2\pi\varrho_{max}^2$$

where r is the radial coordinate in the diffraction plane (x', y'), while ρ is the corresponding radial coordinate in the pupil plane (x, y).

During the calculation of Eq. (3) we have used two of the important properties of the Fourier transformation, i.e., the addition and the shift theorems [6].

Equation (3) can be rewritten in terms of the trigonometric functions to get the PSF as follows:

$$\mathrm{PSF}(W) = aJ_0(W)+\beta\frac{J_1(W/4)}{(W/4)}\cos^2(3W/8). \tag{4}$$

Using CSOM the image of a point object is obtained [2], i.e.,

$$I(W) = |h_o(W)\times h_c(W)|^2 \tag{5}$$

and

$$h_r(W) = h_o(W)\times h_c(W)$$

where h_0 is the PSF for the objective lens, h_c – for the collector, and h_r is the PSF for the resultant. Assuming equal pupils of the objective and the collector, and substituting Eq. (4) to Eq. (5) we get

$$I(W) = \mathrm{const}\left\{J_0(W)+\gamma\frac{J_1(W/4)}{(W/4)}\cos^2(3W/8)\right\}^4 \tag{6}$$

where $\gamma = \beta/a = 1/16$ and $\mathrm{const} = 16\pi^4\varrho_{max}^8$.

Now, we shall calculate the PSF for another type of pupils consisting of an external annulus, internal annulus, set of four equal circles situated along the axes x, y, and of another set of four equal circles, which are found along the axes rotated by an angle of 45° with respect to the axes x, y (Fig. 2).

The pupil under study has eight-fold symmetry and it can be represented mathematically as follows:

$$P(\rho) = \delta(\rho-\rho_{max})+\delta(\rho-\rho_{max}/4)+\sum_i P_i(\rho)+\sum_l P_l(\rho) \tag{7}$$

where $\delta(\rho-\rho_{max})$ is the pupil function for the external anulus, and $\delta(\rho-\rho_{max}/4)$

– for the internal annulus, assuming that the annuli are very thin

$$P_i(\rho) = 1, \text{ for } |\rho-\rho_i| \leqslant \rho_{max}/4 \tag{8}$$

where

$$\begin{array}{llll} \varrho^2 = X^2+Y^2, & \text{for } i = 1, & X = x, & Y = y-y_1, \\ & i = 2, & X = x, & Y = y+y_1, \\ & i = 3, & X = X-x_1, & Y = y, \\ & i = 4, & X = x+x_1, & Y = y. \end{array}$$

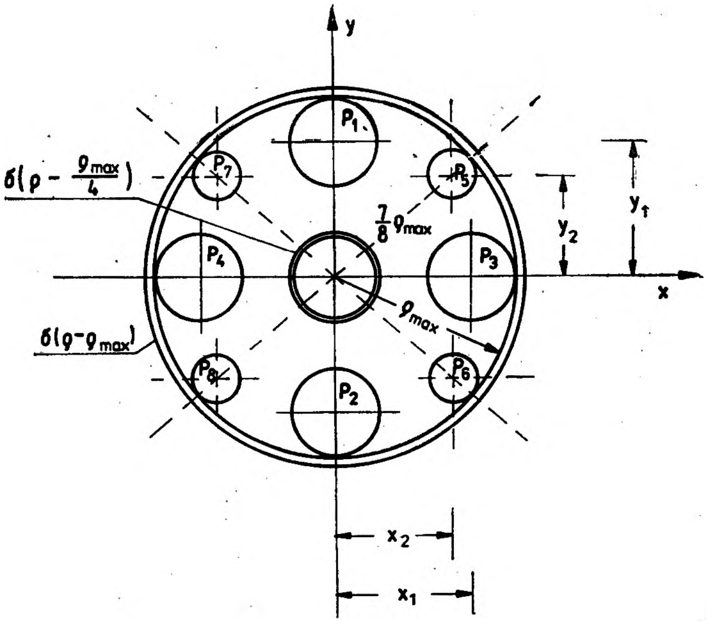

Fig. 2. Pupil consisting of two annuli and two different sets of four equal circular appertures

also

$$P_l(\rho) = 1, \quad \text{for } |\rho-\rho_l| \leqslant \rho_{max}/8 \tag{9}$$

where:

$$\begin{array}{lll} \text{for } l = 5, & X = x-x_2, & Y = y-y_2, \\ l = 6, & X = x-x_2, & Y = y+y_2, \\ l = 7, & X = x+x_2, & Y = y-y_2, \\ l = 8, & X = x+x_2, & Y = y+y_2. \end{array}$$

Now, we apply the two-dimensional Fourier transformation to Eq. (7) and obtain finally this expression for the PSF:

$$\mathrm{PSF}(W) = \alpha' J_0(W)+\beta' J_0(W/4)+\gamma'[J_1(W/4)/(W/4)] \times\left[\cos(3W/4\sqrt{2})+\frac{T}{4}\cos^2(7W/16)\right] \tag{10}$$

where

$$T = \text{const}\,\frac{J_1(W/8)/(W/8)}{J_1(W/4)/(W/4)}, \qquad \gamma' = \frac{\pi}{8}\,\varrho_{\max}^2$$

α', β', and γ' being positive constants depending on the pupil function.

The point image can be calculated from Eq. (10) to give:

$$I(W) = |\mathrm{PSF}(W)|^4,\ I(W) = -|\mathrm{PST}(W)|^4. \tag{11}$$

A computer program has been made to compute the formulae (6) and (11). The results obtained are represented graphically for comparative reason. The theoretical curve corresponding to full circular aperture under the same imaging conditions is given.

It is to be noted that W is also a two-dimensional quantity and $I(W)$ means $I(U, V)$, where U and V are the reduced coordinates in the diffraction plane x, y.

3. Results and discussion

The filter function shown in Fig. 1 was used to represent graphically in Fig. 3 theoretical calculation of formula (6) for the intensity distribution of a point object imaging using CSOM. It is found that the central disc of the diffraction pattern is sharpened giving the cut-off at $r_c = 0.26$ µm if compared to circular aperture calculations (Fig. 5).

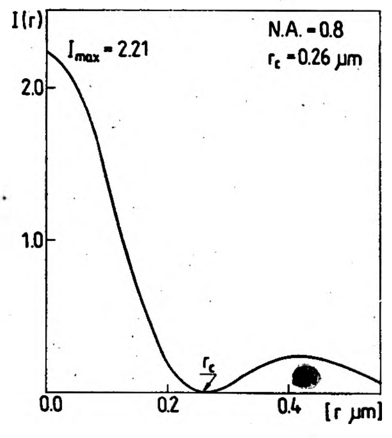

Fig. 3. Image of a point object in confocal scanning optical microscope equipped with pupils (as in Fig. 1) and numerical apertures of $N.A. = 0.8$; r_c is the cut-off of the diffraction pattern

Then, using the second filter function (in Fig. 2) the intensity of a point object imaging was calculated from formula (11) and represented graphically in Fig. 4. The central disc $r_c = 0.18$ µm is much sharper if compared to circular and annular calculations. The first secondary maximum is found at one half of the maximum intensity at the centre (approximately).

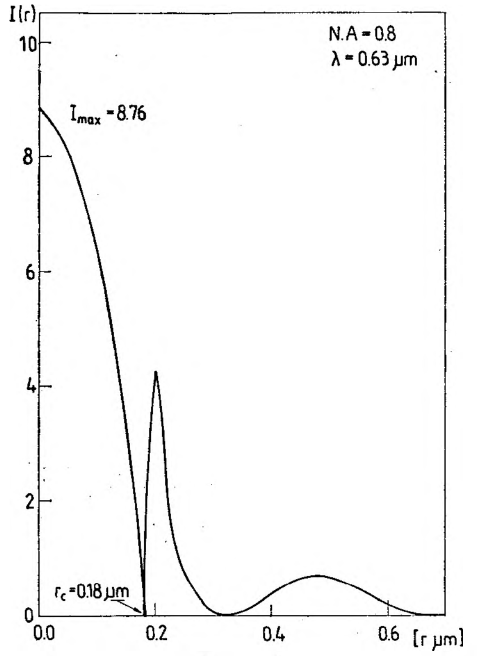

Fig. 4. Image of a point using CSOM equipped with pupils (as in Fig. 2) having numerical apertures of $N.A. = 0.8$

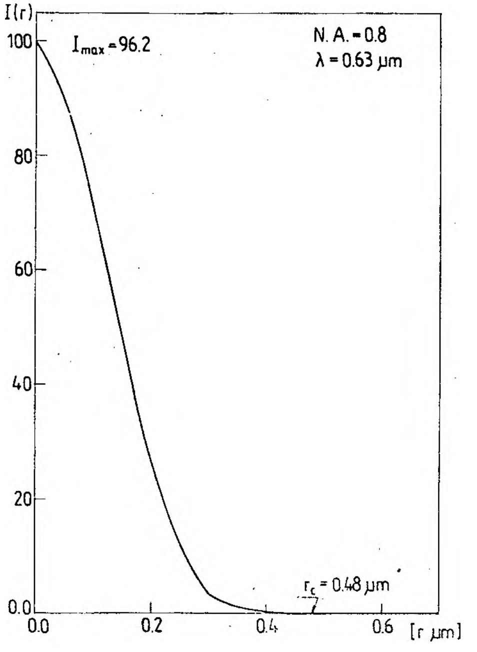

Fig. 5. Image of a point object using CSOM equipped with full circular apertures of $N.A. = 0.8$

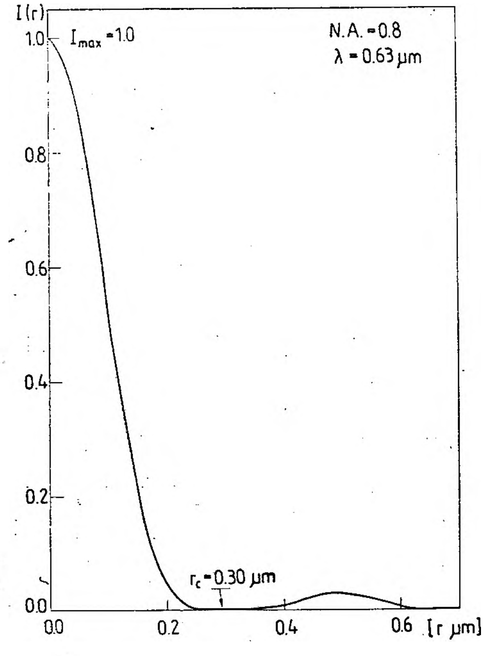

Fig. 6. Image of a point object using CSOM equipped with annular apertures of $N.A. = 0.8$

Finally, using a full circular aperture, the intensity distribution was calculated giving $r_c = 0.48$ μm (Fig. 5), while with an annular aperture the cut-off was at $r_c = 0.30$ μm (Fig. 6).

All the calculations were made under the same imaging conditions using confocal scanning optical microscope (CSOM) equipped with aperture of numerical aperture ($N.A. = 0.8$).

We conclude that the resolution (Figs. 3, 4) is comparable with annular aperture calculations (Fig. 6), but the outer rings of the diffraction pattern being much stronger make our aperture pupil functions useful for practical imaging of extended objects. We have shown theoretically the possibility of improving the microscope resolution at the cost of small degradation of image contrast if compared to annular circular calculations. In reality, the distance between the ideal image point and the first zero of the point spread function is not necessarily a good indicator of resolution for all imaging systems, i.e., the resolution of the optical instrument depends on the overall behaviour of the PSF and not simply on the radius of the first zero r_c.

References

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