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Image and super-resolution in optical coherent microscopes

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We present a hyper-resolvant pupil of linear amplitude distribution for the improvement of microscope resolution. The point spread function of this pupil and the image of a point object have been calculated. Another type of pupils obstructed by a series of black annular diaphragms has been investigated in order to calculate the image of a point. We have also calculated the intensity distribution in an image of a two point object in case of circular pupil obstructed by a series of annuli.

1. Introduction

In the scanning confocal optical microscope, the quality of both lenses of the objective and condenser are equally responsible for the point spread function. The effect of various obstructions with annular pupils was previously given by Sheppard et al. [1].

Recently, we have studied another system of obstructed pupils using lenses having a conic pupil function. We have shown an improvement of the shape of the resultant point spread function [2].

In this paper super resolution pupils are described by constructing objective lenses with a linear amplitude as a function of the pupil radius. These pupils will give the best resolution as we shall discuss in the following Sections. Obstructed circular pupils having an alternative finite number of black and transparent annular zones to calculate the image of a point object are also presented. The two point resolution is given.

2. Analysis

2.1. Calculation of the image of a point object for a pupil with linear amplitude distribution

The problem in two dimensions is solved by using polar coordinates. The linear amplitude distribution for the pupil under consideration can be represented as follows:

$$P(\varrho) = |\varrho/\varrho_0| \text{ if } |\varrho/\varrho_0| \leqslant 1. \tag{1}$$

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This pupil is considered as a hyper-resolvant pupil because of its transmission distribution. It offers the advantage of the attenuation of low frequency and, consequently, the diffracting object structure is imaged with enhanced contrast because object areas of slowly varying transmission is attenuated [3]. Therefore we believe that the low spatial frequency of the image is attenuated which also gives better contrast of higher frequency components of the image spectrum.

Returning now to the formula (1), and applying the two dimensional Bessel-Fourier transform, we can write the point spread function as follows:

$$h_1(r) = 2\int_0^{2\pi}\int_0^1 \varrho \exp\left(-\frac{j2\pi}{\lambda}\frac{\varrho r}{f}\cos\Theta\right)\varrho d\varrho d\Theta = 4\pi\int_0^1 \varrho^2 J_0(k\varrho r/f)d\varrho \tag{2}$$

where $k = 2\pi/\lambda$ is the propagation constant.

We have obtained the impulse response for the objective lens

$$h_1(w) = 4\pi(f/kr)^3\int_0^W wJ_0^2(w)dw \tag{3}$$

with $W = kr/f$, and $w = k\varrho r/f$.

The solution of integral (3) is given by HAMED [3] during the treatment of conic amplitude distribution as a pupil function. The result leads to

$$h_1(w) = 4\pi(f/kr)^3\left[W^2J_1(W)+WJ_0(W)-2\sum_i J_i(W)\right] \tag{4}$$

(with $i = 1, 3, 5, \ldots$).

Hence, the impulse response of one pupil can be rewritten to give

$$h_1(W) = 4\pi\left[J_1(W)/W+J_0(W)/W^2-2\sum_i J_i(W)/W^3\right]. \tag{5}$$

Consequently, the resulting impulse response of the optical system in a confocal microscope yields

$$\begin{aligned} h_T(W) = h_1(W)h_2(W) = 16\pi^2\Big[&\{J_1(W)/w\}^2+\{J_0(W)/W^2\}^2+ \\ &+2J_0(W)J_1(W)/W^3+4\Big\{\sum_i J_i(W)/W^3\Big\}^2-4\{J_1(W)/W+ \\ &+J_0(W)/W^2\}\sum_i J_i(W)/W^3\Big]. \end{aligned} \tag{6}$$

Hence the intensity spread function gives

$$I(W) = |h_1(W)h_2(W)|^2 = |h_T(W)|^2.$$

It is to be noted that formula (6) assumes two symmetric pupils, hence

$$I(W) = 256\,\pi^4\left[J_1(W)/W+J_0(W)/W^2-2\sum_i J_i(W)/W^3\right]^4. \tag{7}$$

In the case of two points separated by a distance W_1, the image can be calculated easily to give

$$I(W) = |[\delta_1(W)+\delta_2(W\pm W_1)]*h_T(W)|^2 = |h_T(W)+h_T(W\pm W_1)|^2 \tag{8}$$

where $*$ denotes the convolution operation.

In the case of circular pupil for the objective lens, and linear amplitude distribution for the collector lens, or vice versa, we have got the following total impulse response of the optical system:

$$h_T(W) = 8\pi\{J_1(W)/W\}\left[J_1(W)/W+J_0(W)/W^2-2\sum_i J_i(W)/W^3\right], \tag{9}$$

$$I(W) = |h_T(W)|^2. \tag{9a}$$

In the case of annular pupil with the former pupil which exhibits radially increasing linear amplitude distribution, we have got

$$h_T(W) = \mathrm{const}\,J_0(W)\left[J_1(W)/W+J_0(W)/W^2-2\sum_i J_i(W)/W^3\right]. \tag{10}$$

Consequently, the image of a point object, in this case, gives

$$I(W) = \mathrm{const}\,J_0^2(W)\left[J_1(W)W+J_0(W)/W^2-2\sum_i J_i(W)/W^3\right]^2. \tag{11}$$

It is to be noted that computer programs are constructed to compute numerically the formule (7), (9a) and (11).

2.2. Obstructed circular pupil with black and transparent equal areas

Referring to Figure 1, we have a circular pupil having numerical aperture ($N.A. = n'\sin\Theta \simeq \varrho/f$) where Θ is the half-angular aperture of the lens under consideration and n' is the refractive index of the object medium (in this case $n' = 1$).

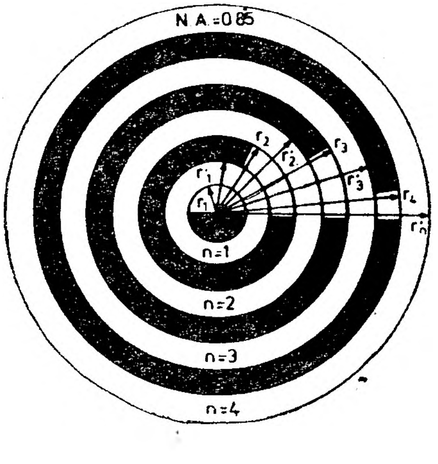

Fig. 1. Circular pupil obstructed with a finite number of successive black and transparent areas

The point spread function for a pupil of this form can be easily calculated analytically, if we take into consideration that each zone is computed from the difference between two consecutive circular pupils

$$h_1(w) = F.T.\ \{P(B/W)\}, \tag{12}$$

where B/W denotes the succession of black and white (transparent) areas $F.T.$ is the Fourier transform operation.

The Equation (12) can be rewritten as follows:

$$h_1(w) = F.T. \sum_{n=1}^{N} P_{r_n} = F.T.\{\Delta P_{r_1} + \Delta P_{r_2} + \ldots + \Delta P_{r_i}\} \tag{13}$$

where, e.g., $\Delta P_{r_1} = P_{r_1'} - P_{r_1}$ (Fig. 1), and N is the total number of transparent zones constituting the whole pupil.

Hence, the Fourier transformation over (13), gives for the point spread function the expression below

$$h_1(w) = \sum_{n=1}^{N} \left\{ r_n'^2 \left[\frac{2J_1(w_n')}{w_n'}\right] - r_n^2 \left[\frac{2J_1(w_n)}{w_n}\right] \right\} \tag{14}$$

where $w = (2\pi r/\lambda f)\varrho$; λ is the wavelength of the illumination, f – the focal length of the lens, and r – the radial coordinate in the object plane. This expression (14) is valid for whatever the number of annuli constituting the pupil.

Hence, by considering two symmetric pupils of the above type satisfying formula (13), we can find that the resulting point spread function for the optical coherent confocal microscope is expressed by general formula

$$h_T(w) = h_1(w)h_2(w) = \sum_n \left\{ r_n'^2 \left[\frac{2J_1(w_n')}{w_n}\right] - r_n^2 \left[\frac{2J_1(w_n)}{w_n}\right] \right\} +$$
$$+ \sum_n \sum_{m \neq n} \left\{ \left(r_n'^2 \left[\frac{2J_1(w_n')}{w_n'}\right] - r_n^2 \left[\frac{2J_1(w_n)}{w_n}\right] \right) \left(r_m'^2 \left[\frac{2J_1(w_m')}{w_m'}\right] - r_m^2 \left[\frac{2J_1(w_m)}{w_m}\right] \right) \right\}. \tag{15}$$

Consequently, the image for a point object in this case is

$$I(w) = |h_T(w)|^2, \tag{16}$$

while the image of two points separated by a distance w_1 was given previously by formula (8), taking into consideration that here $h_1 h_2$ is given by the Eq. (15).

3. Results and discussion

We calculate numerically the point spread function for the optical coherent confocal microscope in all cases cited previously in the analysis. The image of a point object is calculated, too. The wavelength of light is taken $\lambda = 0.6328$ μm.

The calculations are made using high numerical aperture for the two objectives ($N.A. = 0.85$), and repeated for lower $N.A. = 0.25$, and 0.5.

The image of a point was calculated numerically by using two symmetric pupils for the objective lenses with linear pupil transmission. In the case of two different pupils where one of them is circular and the second has linear transmission, the point spread function is also calculated by using formula (9a). A combination with annular pupil is also made to calculate the image of a point using Eq. (11).

The results are given graphically in Figure 2, where the curve 1 corresponds

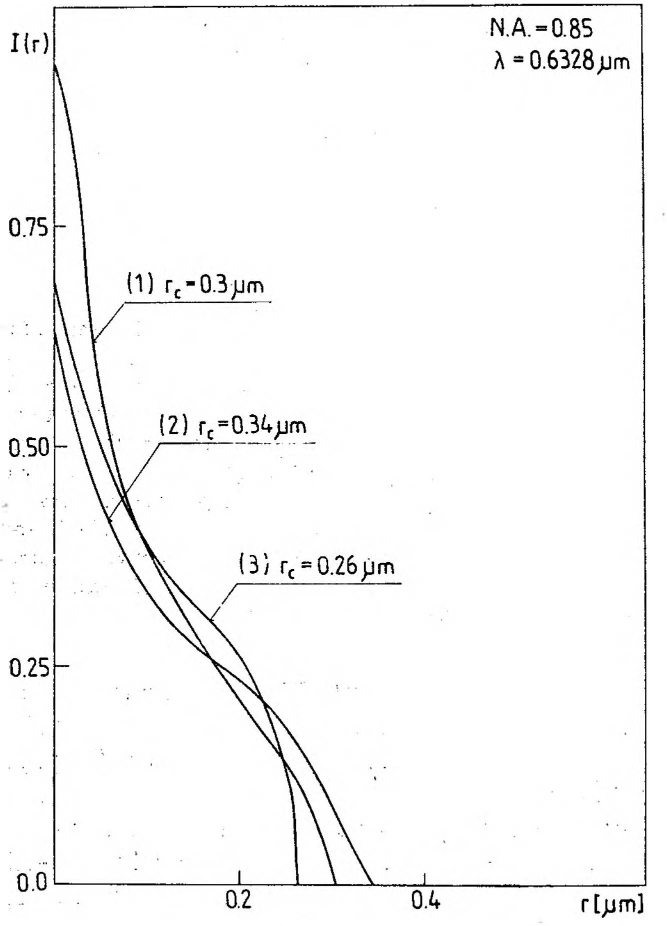

Fig. 2. Image of a point object using coherent confocal microscope. Curve 1 is given for two symmetric pupils with linear amplitude distribution, curve 2 is given for the former pupil associated with another circular pupil, and the last curve 3 is constructed for the former pupil associated with annular pupil. r_c is the first minimum of diffraction pattern corresponding to each curve

to the results of the formula (7), giving the radius of the first minimum of diffraction pattern at $r_c = 0.3$ μm, while the curve 2 is obtained by the formula (9) and the last curve 3 corresponds to the formula (11), giving $r_c = 0.26$ μm.

From the results of calculations it follows that improvement of resolution is at the expense of the contrast of the image, i.e., when we try to improve the resolution by certain means of obstruction, the contrast of the image decreases.

In this work, we have chosen a pupil having linear amplitude distribution, which is considered as a super-resolvant pupil. This pupil has improved both the limit of resolution of the confocal coherent microscope, giving r_c at 0.3 μm, and the contrast of the image, as was expected (Fig. 2, curve 1).

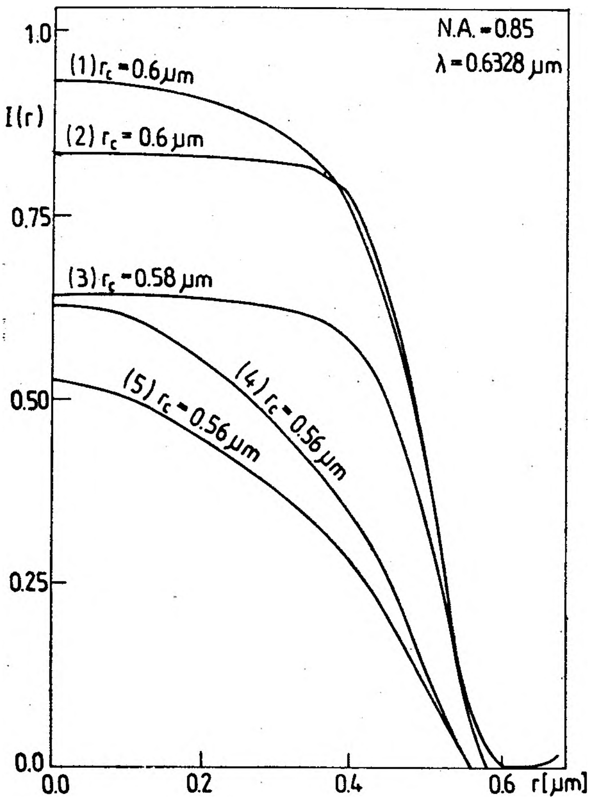

Fig. 3. Image of a point object for the microscope under consideration. Curves 1, 4 and 5 are given by using four successive black and transparent areas with the ratios 1.25:1:1:1, 4.5:1:1:2, and 4.5:1:1:1, respectively. Curve 2 is given for six equal black and white areas, and curve 3 corresponds to eight black and white equal areas

Another set of curves, giving the image of a point object for two symmetric pupils which have the same $N.A.$ and then contains a finite number of annular zones of equal width is presented in Fig. 3. Curve 1 is given for a circular pupil having four equal zones of which two are transparent. Curves 4 and 5 are also constructed for the same number of zones, but with the respective ratios: 4.5:1:1:2 and 4.5:2:1:1. The last two curves 4, 5 give better resolution with respect to curve 1, due to the appropriate selection of the areas constituting the pupil. Curve 2 is given also for six equal annular zones giving $r_c = 0.6$ μm, while curve 3 is constructed for eight equal zones giving r_c at 0.58 μm, $r_c = 0.56$ μm for the curves 4 and 5.

Figure 4 gives the image of a two point object using the pupils with three transparent annular zones. This figure is graphically constructed after the numerical computation of the formulae (15) and (16). We can see from the results of Fig. 4 that the contrast of the image, which is given by the experimental definition of the visibility $V = (I_{max} - I_{min})/(I_{max} + I_{min})$, increases when the distance between the two point object increases.

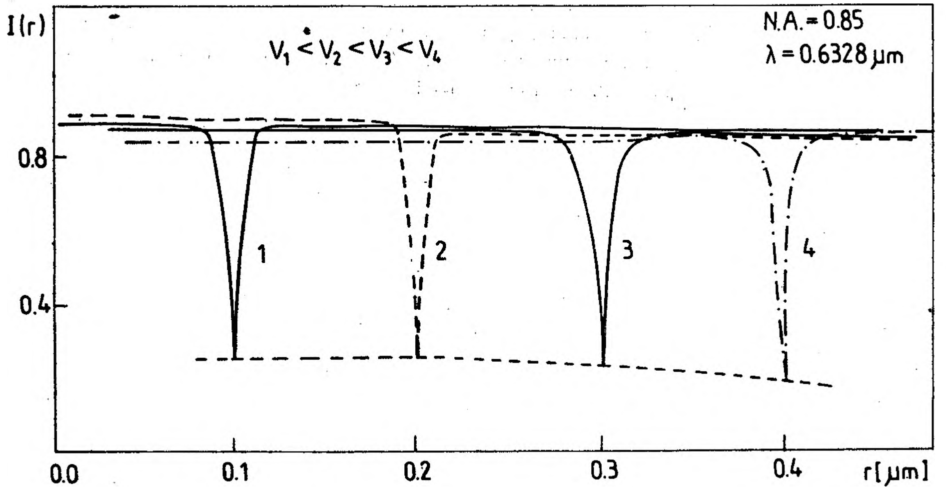

Fig. 4. Image of a two point object using coherent confocal microscope with circular obstructed pupils having six equal black and transparent areas. The distances between the two points were: 0.1, 0.2, 0.3 and 0.4 μm, respectively, and the contrast of the image is given by the definition of the visibility $V = (I_{max} - I_{min})/(I_{max} + I_{min})$

4. Conclusions

We conclude from the results obtained that the super-resolution pupil which has a linear amplitude distribution, improves the resolution of the confocal coherent microscope and enhances the contrast of the image simultaneously. This improvement of resolution was about 20% with respect to the circular pupil calculation which gives the first minimum of diffraction pattern at $r_c = 0.37$ μm. Another improvement of resolution about 30% has been obtained with our pupil associated with annular pupil.

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Образ с ультрараспределением в когерентном оптическом микроскопе

Представлен сверхраспределительный зрачок с линейным распределением амплитуды, примененный для повышения разделительных свойств микроскопа. Рассчитана пунктирная функция размывания зрачка изображением пунктирного предмета. Исследован, кроме того, другой тип зрачков с диафрагмами в виде серии черных кольцеобразных диаграмм, с целью рассчета изображения пункта. Рассчитано также распределение напряжения в изображении двухпунктирного предмета в случае кругового зрачка, диафрагмированного серией колец.

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