Optica Applicata, Vol. XV, No. 1, 1985

Letters to the Editor

Remarks on point spread function in confocal scanning microscope with apodized pupil*

ANNA MAGIERA, LEON MAGIERA

Institute of Physics, Technical University of Wrocław, Wybrzeże Wyspiańskiego 27, 50–370 Wrocław, Poland.

The properties of confocal microscope were first discussed by MINSKY [1] and investigated by EGGER et al. [2] and DAVIDOVITZ et al. [3]. The scheme of this microscope is presented in Fig. 1. The image intensity distribution in such a microscope is given by [3]

$$I' = |t*(h_1h_2)|^2 \tag{1}$$

where t is the object amplitude transmittance, h_1 and h_2 are amplitude point spread functions of objective and collector, respectively, and $*$ denotes convolution. From Equation (1) we see that confocal scanning microscope has the

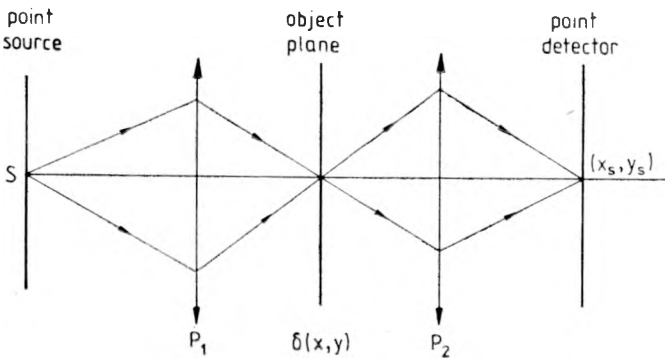

Fig. 1. Scheme of confocal scanning microscope

properties of conventional coherent microscope, the point spread function being the product of h_1 and h_2. Therefore, the effective intensity point spread function (EPSF) has the form

$$I' = |h_1h_2|^2. \tag{1a}$$

The central peak in this EPSF is sharpened up and the side lobes are diminuted in comparison with the conventional point spread function for a uniform pupil.

To improve the EPSF Sheppard proposed to use an annular pupil in the confocal microscope [4]. The intensity point spread function for annular pupil

* This work was carried on under the Research Project M.R. I.5.

has the form

$$I(z) \sim \frac{1}{(1-\varepsilon^2)^2}\left[\frac{2J_1(z)}{z} - \varepsilon^2 \frac{2J_1(\varepsilon z)}{\varepsilon z}\right]^2 \tag{2}$$

where ε means the ratio of radii of annulus, J_1 the Bessel function of the first order. The minima of this diffraction pattern are the solutions of

$$J_1(z) - \varepsilon J_1(\varepsilon z) = 0. \tag{3a}$$

For a proper obscuration ε, the minima of the point spread function of the annulus coincide with the maxima of the point spread function corresponding to the circular pupil. In this case side lobes of the effective point spread function are extremely small, the central peak being narrower. The narrowest central peak $z = 2.4$ corresponds to $\varepsilon \to \infty$.

A further improvement of the resolution in the confocal scanning microscope suggested in this paper can be achieved if one of the two pupils applied gives the point spread function with a prescribed localization of zeros. Such a pupil has the form of a linear combination of Bessel functions [5]

$$f(r) \sim 1 - \sum_{i=1}^{M} b_i J_0(z_i r) \tag{3b}$$

where z_i's are the prescribed zeros. M is the number of specified zeros, J_0 denotes the zero-order Bessel function, and coefficients b_i satisfy the following set of linear equations:

$$\sum_{j=1}^{M} b_j L(z_j, z_i) = \int_0^1 J_0(z_i r) r dr, \; i = 1, 2, \ldots, M$$

with (4)

$$L(z_j, z_i) = \int_0^1 J_0(z_i r) J_0(z_j r) r dr.$$

The point spread functions of objective and collector can be calculated by Fourier transformations of their pupils. Therefore, the EPSF for the combination of one uniform pupil and the other of the form (4) writes

$$I'(z) \sim \left[\frac{J_1(z)}{z}\right]^2 \left[\frac{J_1(z)}{z} - \sum_{i=1}^{M} b_i L(z_i, z)\right]^2. \tag{5}$$

The first and second factors of (5) correspond to uniform and apodized (3) pupils, respectively.

The obtained numerical results are presented in Figs. 2, 3 and 4. The number of zeros and their localizations may be read out from the Table. In Figure 2 the point spread functions of conventional microscope are drawn for different sets of zeros (curves 2, 3). In Figures 3 and 4 the following curves are plotted:

– point spread function for conventional microscope with uniform pupil (curve 1),

– EPSF for uniform pupils (curve 2),

– EPSF for one uniform- and one apodized pupil of the form (3).

From the obtained results it can be seen that the proper choice of z_i values gives especially narrow EPSF (Fig. 3a, curve 3), as in this case the relatively

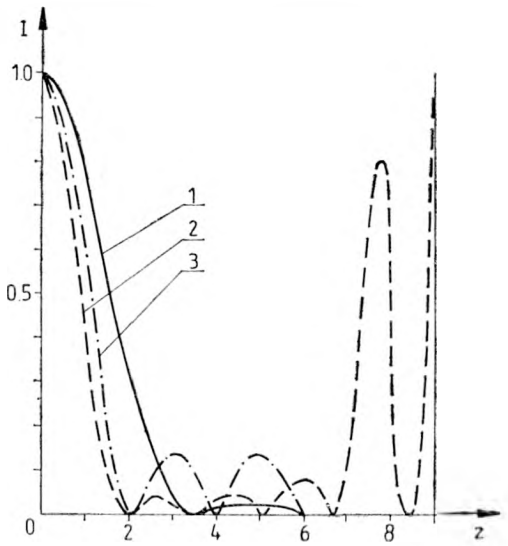

Fig. 2. Point spread functions for conventional microscope with uniform pupil (1), apodized pupils with $M = 5$ (2) and $M = 3$ (3)

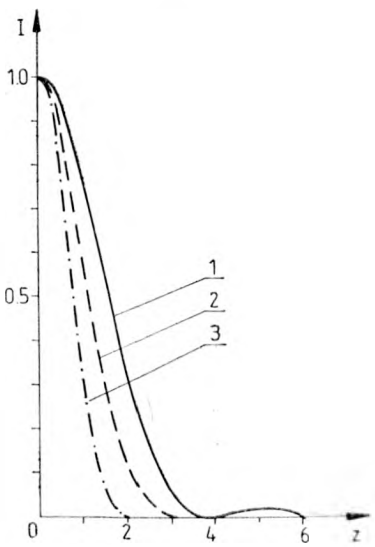

Fig. 3a

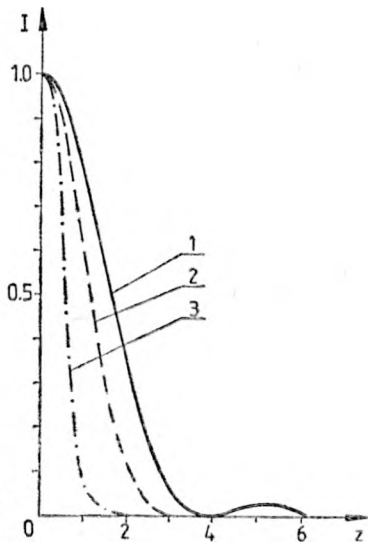

Fig. 3b

Fig. 3a. Point spread functions for microscope: 1 – conventional microscope with uniform pupil, 2 – confocal microscope with uniform pupils, 3 – confocal scanning microscope with one uniform pupil and the second apodized one, with $M = 5$

Fig. 3b. The same as in Fig. 3a, curve 3 corresponds to $M = 3$

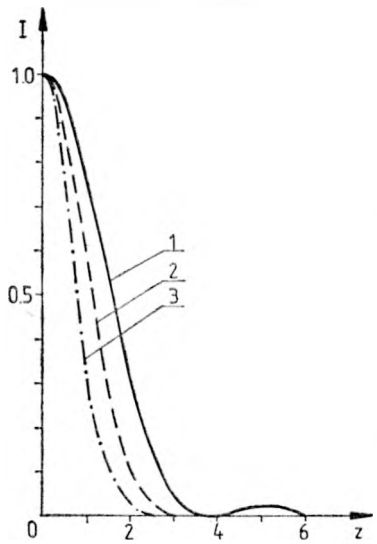

Fig. 4a

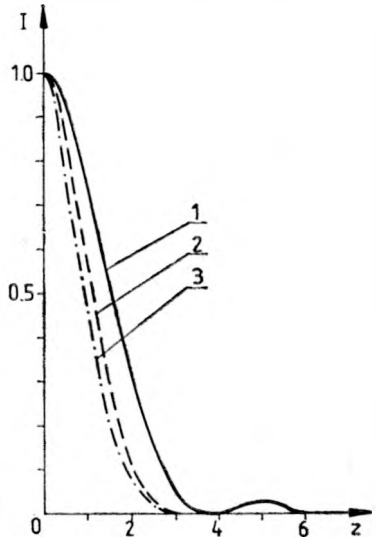

Fig. 4b

Fig. 4a. The same as in Fig. 3a, curve 3 corresponds to $M = 1$, and $z_1 = 2.5$
Fig. 4b. The same as in Fig. 3a, curve 3 corresponds to $M = 1$ and $z_1 = 3$

M	5	3	1	1
z_1	1.91585	1.91585	2.5	3
z_2	3.5078	4.1		
z_3	5.08675	5.9		
z_4	6.66185			
z_5	8.23532			

great secondary maximum is localized in the region in which the point spread function for conventional microscope with uniform pupil is practically negligible (Fig. 2, curves 1 and 2). We hope the proper choice of M and z_i will give even better results.

References

[1] MINSKY M., U.S. Patent 3013467, *Microscopy apparatus*, Dec. 19, 1961 (filed Nov. 7, 1957).
[2] EGGER M. D., PETRÁŇ M., Science **157** (1967), 305.
[3] DAVIDOVITZ P., EGGER M. D., Nature **223** (1969), 831.
[4] SHEPPARD C. J. R., WILSON T., Optik **55** (1980), 331.
[5] BOIVIN R., BOIVIN A., Optica Acta **27** (1980), 587.

Received June 30, 1984