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Letter to the Editor

Axial intensity response of the confocal two-photon fluorescence microscopy with apodization

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The effect of falling off with the fourth power of u of the axial intensity response in the confocal two-photon fluorescence microscopy has been restricted by applying suitable apodizers. Critical Rayleigh axial resolutions were determined.

1. Introduction

Two-photon fluorescence microscopy was first proposed by SHEPPARD and KOMFNER [1]. In paper [2], the radial and axial image intensity responses for two-photon fluorescence, confocal two-photon fluorescence, confocal fluorescence and conventional fluorescence microscopies were compared. The confocal two-photon fluorescence microscopy offers lower radial resolution than the conventional fluorescence microscopy, while the axial resolution is substantially lower in the first case compared with the latter. However, the sectioning property of the confocal two-photon fluorescence mode appears to be very sharp for large u; the axial intensity response falling off as the fourth power of u. In this paper, the axial intensity response of the confocal two-photon fluorescence microscopy with apodization and a circular detector is investigated.

2. Theory and numerical results

The axial intensity $I(u)$ in a confocal two-photon fluorescence microscopy can be obtained by integrating the three-dimensional intensity distribution generated by an object point, as given by the following formula [3]:

$$I_{C2P}(u) = \int_0^\infty I^2(u/2, v/2) I(u,v) v dv \tag{1}$$

where: $I(u,v)$ – image of a point-source in an aberration-free lens [4],
u,v – axial and radial normalized optical coordinates given by:

$$u = 4kz\sin^2(\alpha/2), \quad v = kr\sin\alpha, \quad k = 2\pi/\lambda,$$

$\sin\alpha$ – numerical aperture of the lens,
z – defocused distance of the object,
r – radius vector in the image plane.

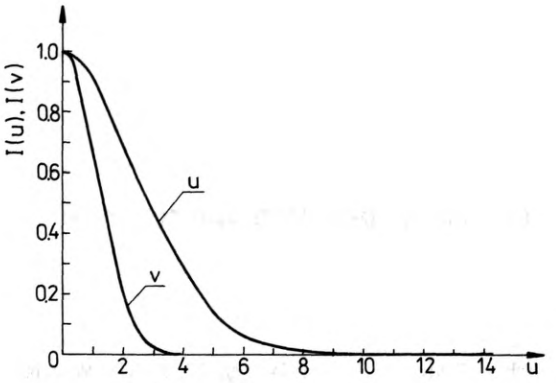


Fig. 1. Axial $I(u)$ and radial $I(v)$ responses of the intensity confocal two-photon fluorescence microscope (C2P)

In Figure 1, the radial $I(v)$ and axial $I(u)$ intensity responses are shown for the case of confocal two-photon fluorescence microscopy.

Assuming the rotational symmetry of the optical system, the image of a point object given by an aberration-free lens with an apodizer $T(\rho)$ is given by

$$I(u,v) = \left| 2\int_0^1 T(\rho) J_0(v\rho) \exp\left(\frac{1}{2} iu\rho^2\right) \rho d\rho \right|^2 \tag{2}$$

where: J_0 – Bessel function of the first kind and zero order,

$T(\rho)$ – transmittance of the amplitude apodizer.

Substituting Equation (2) into (1) and taking account of the fact that a circular detector given in the form of a finite-size pinhole of radius r_d is used in the image plane, the generalized axial intensity response (detected axial signal intensity) $I(u)$ can be obtained for the confocal two-photon fluorescence microscopy in the form

$$I(u) = \int_0^{v_d} \left\{ \left[\int_0^1 T(\rho) \cos\left(\frac{u}{2}\rho^2\right) J_0\left(\frac{v}{2}\rho\right) \rho d\rho \right]^2 + \left[\int_0^1 T(\rho) \sin\left(\frac{u}{2}\rho^2\right) J_0\left(\frac{v}{2}\rho\right) \rho d\rho \right]^2 \right\}^2$$

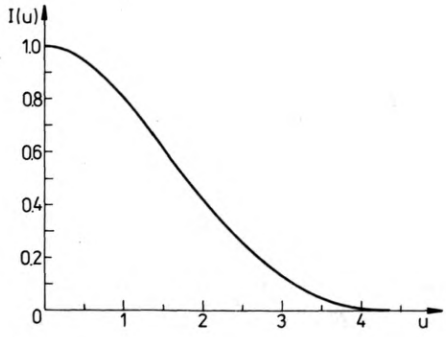
$$\times \left\{ \left[\int_0^1 T(\rho) \cos(u\rho^2) J_0(v\rho) \rho d\rho \right]^2 + \left[\int_0^1 T(\rho) \sin(u\rho^2) J_0(v\rho) \rho d\rho \right]^2 \right\} > v dv, \tag{3}$$

with $v_d = \frac{2\pi}{\lambda} r_d \sin\alpha$ being the normalized radius of the detector, and the following types of apodizers being chosen for numerical calculations:

$$T(\rho) = \begin{cases} 1-\rho^2 & |\rho| \leqslant 1, \\ 0 & |\rho| > 1, \end{cases}$$

$$T(\rho) = \begin{cases} 1/2(1+\rho^2) & |\rho| \leqslant 1, \\ 0 & |\rho| > 1, \end{cases}$$

$$T(\rho) = \begin{cases} \rho^2 & |\rho| \leqslant 1, \\ 0 & |\rho| > 1. \end{cases}$$



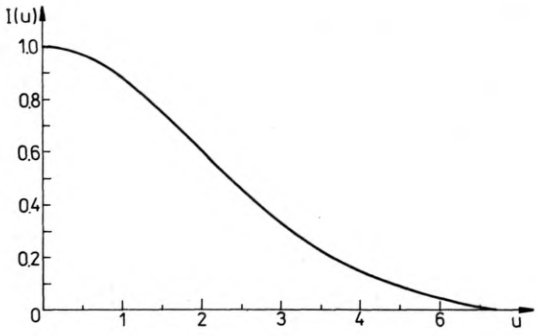


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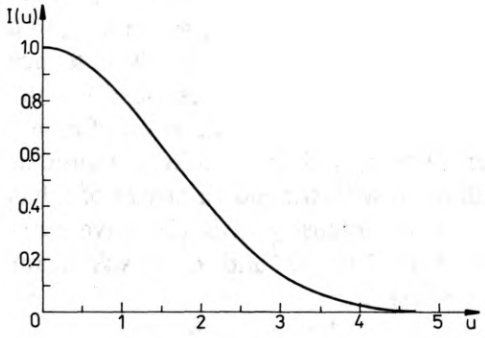
Fig. 2. Axial response of the intensity $I(u)$ when normalized radius of the detector $v_d = 1$

Fig. 3. Axial image response of the intensity $I(u)$ microscope when $v_d = 1$, $T(\rho) = 1-\rho^2$



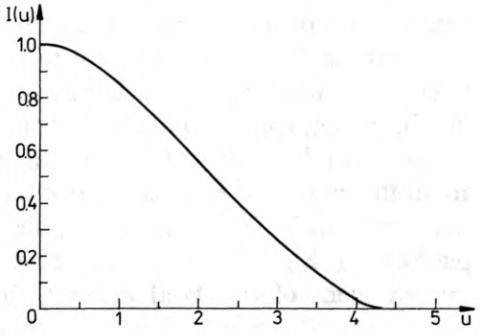


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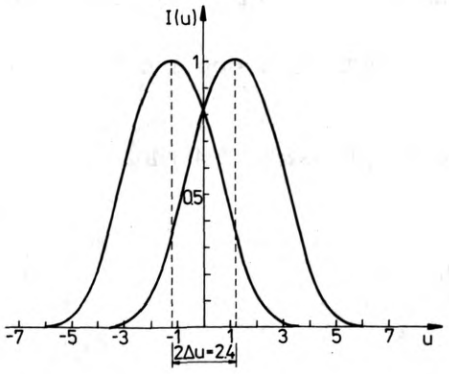
Fig. 4. Axial response of the intensity $I(u)$ microscope when $v_d = 1$, $T(\rho) = 1/2(1+\rho^2)$

Fig. 5. Axial response of the intensity $I(u)$ microscope when $v_d = 1$, $T(\rho) = \rho^2$



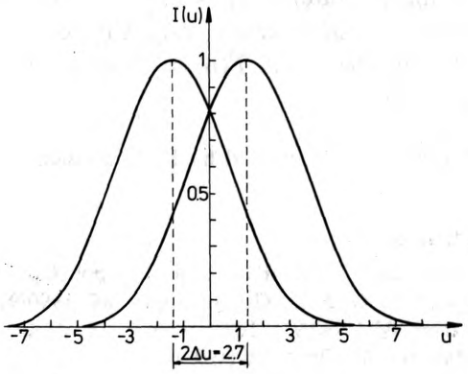


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Fig. 6. Two-point Rayleigh resolution for non-apodized C2P microscope

Fig. 7. Two-point Rayleigh resolution for C2P microscope with apodization $T(\rho) = (1-\rho^2)$

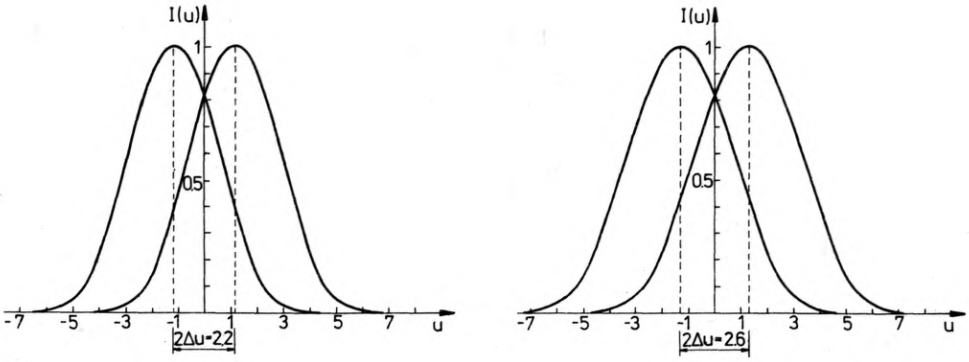

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Fig. 8. Two-point Rayleigh resolution C2P microscope with apodization $T(\rho) = 1/2(1+\rho^2)$
Fig. 9. Two-point Rayleigh resolution C2P microscope with apodization $T(\rho) = \rho^2$

For the sake of comparison with the results reported in papers [3] and [4], the same values of the normalized radius of the pinhole detector, *i.e.* $v_d = 1$ and $v_d = 6$ were chosen. The axial intensity response of the confocal two-photon fluorescence microcope, when $v_d = 1$, is shown in Fig. 2. The axial intensity response for the apodized confocal two-photon fluorescence microscope and the same value of $v_d = 1$ is shown in Figs. 3–5 for different apodizers. For $v_d = 6$ the results are similar. In all the cases, the effect of axial intensity falling off with the fourth power of u has been restricted. The truncation points of the axial intensity response have been marked in Figs. 3–5. In the case presented in Figs. 2 and 6, a significant improvement of the axial intensity has been achieved.

A moderate improvement was obtained in the cases shown in Figs. 4 and 8, and 5 and 9, while only a slight betterment may be observed in Figs. 3 and 7. In accordance with the Rayleigh criterion, two object points are just resolved if the intensity in the middle point between the images is equal to $0.82I_0$. From the results presented in Figs. 6–9, it follows that for the apodizer of $T(\rho) = 1/2(1+\rho^2)$ type resolution is better ($2\Delta u \sim 2.2$) than for nonapodized confocal two-photon fluorescence microscope ($2\Delta u = 2.4$), Fig. 6.

The apodizers of $T(\rho) = \rho^2$ and $T(\rho) = 1-\rho^2$ type show opposite behaviour (Figs. 7, 9).

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