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Cut-off spatial frequency in a confocal microscope with trigonometric filters in its pupil

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In this paper characteristics of the cut-off spatial frequency r_c(NA) for different values of the numerical aperture (NA) are shown for a confocal microscope (CSM) with the aperture modulated by filters of $\cos(N\rho)$ and $\cos^2(N\rho)$ types, respectively.

1. Introduction

In confocal scanning microscopes (CSM) the attainable resolving power is higher than that in conventional microscopes with one objective. The resolving power of CSM can still be improved applying apodisation filters (*i.e.*, ring apertures which, however, diminish the contrast) in the pupil of the objective. In the paper [1] it has been shown how the filters of ρ^n type ($n = 2, 4, ..., 16$) improve the resolving power. The distribution of the point spread function (PSF) versus the spatial frequency r in a confocal scanning microscope with the aperture modulated by the filters of ρ^n type ($n = 2, 4, ..., 16$) for the numerical aperture NA = 0.5, 0.8 is also presented in [1]. The cut-off frequencies for numerical aperture NA = 0.5 as dependent on parameters n, $r_c(n)$ have been determined and the characteristics of the cut-off frequencies versus the numerical aperture r_c(NA) have been shown. When applying this type of filters to CSM the resolving power 0.27 μm has been achieved for the numerical aperture NA = 0.8 and $r_c = 0.43$ μm for NA = 0.5 for great values of the parameter n as compared to r_c for the nonmodulated circular aperture ($n = 0$) when NA = 0.5, $r_c = 0.772$ μm and for NA = 0.8, $r_c = 0.483$ μm for $f = 1$ μm, $\lambda = 0.6328$ μm.

In the paper [2] another character of spatial frequency characteristic $r_c(N)$ has been shown as dependent on the parameter N in the CSM with the aperture modulated by the filters of $\cos(N\rho)$ type for the numerical apertures NA = 0.2, 0.5, 1.0. The characteristics $r_c(N)$ manifest oscillations (which is not observed for the apertures modulated by the filters of ρ^n type for $n = 2, 4, ..., 16$) while the number of oscillations for greater numerical aperture (NA = 1.0) is higher than the number of oscillations for NA = 0.2. With the increase of $N = 0, ..., 20$ the value of r_c may take greater or smaller values than that for r_c corresponding to the nonmodulated aperture ($N = 0$) and $\lambda = 0.6328$ μm.

In this paper the characteristics of cut-off frequencies r_c as dependent on the numerical aperture NA = 0.05, 0.1, ..., 1.2 in CSM with the aperture modulated by the filters of $\cos(N\rho)$ type for the parameter $N = 1.0, 4.5, 7.5, 12$ and the aperture modulated by the filters of $\cos^2(N\rho)$ type for the parameter $N = 1.0, 2.5, 5.0, 7.0, 12.0, 15.0$ have been shown.

2. Numerical calculations

It is well known that the PSF is a Fourier transform of the pupil function $P(\rho)$, *i.e.*, PSF = F.T.$\{P(\rho)\}$. For the apertures modulated by trigonometric filters we obtain

$$h_N = 2\pi \int_0^{\rho_0} A^{**} \rho J_0\left(\frac{k\rho r}{f}\right) \mathrm{d}\rho$$

where: A^{**} – $\cos(N\rho)$, $\cos^2(N\rho)$, J_0 – Bessel function of first kind and zero order, ρ_0 – maximum value of ρ (ρ being the absolute value of a radius vector in the pupil plane), $k = 2\pi/\lambda$ – propagation constant.

For the numerical calculations it has been assumed that $f = 1$ µm, $\lambda = 0.6328$ µm and step $\Delta N = 0.05$ while in the vicinity of discontinuity the step has been reduced to $\Delta N = 0.001$. The cut-off spatial frequencies r_c have been determined solving the equation $h_N = 0$.

In Figure 1, the characteristic r_c(NA) in CSM with the nonmodulated aperture ($N = 0$) is shown. The cut-off frequency r_c (µm) for different values of NA in CSM with the aperture modulated by filters of $\cos(N\rho)$ type is shown in Figs. **2a**–**d** while that for the aperture modulated with filters of $\cos^2(N\rho)$ type in Figs. 3**a**–**f**.

For the nonmodulated aperture the cut-off frequency in CSM changes from $r_c = 19.3004$ µm for NA = 0.02 to $r_c = 0.386$ µm for NA = 1.0 (see Fig. 1). In CSM modulated by the filters of $\cos(N\rho)$ type for parameter $N = 1$ the cut-off frequency changes from $r_c = 7.71$ µm for NA = 0.05 to $r_c = 0.371$ µm for NA = 1.2 (Fig. 2**a**). For the parameter $N = 4.5$ the cut-off frequency takes its minimum value $r_c = 0.00177$ µm for the numerical aperture NA = 0.518 (Fig. 2**b**). For $N = 7.5$ the cut-off frequency

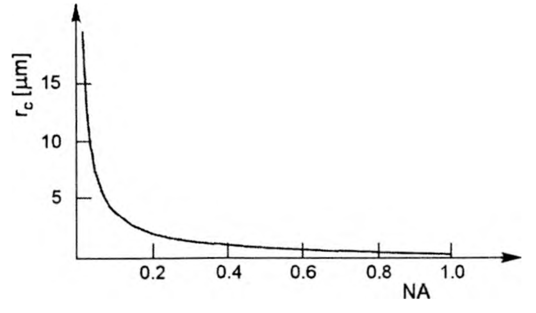

Fig. 1. Cut-off spatial frequencies r_c for different values of the numerical aperture NA = 0.05, 0.1, ...,1.0 in a nonmodulated CSM.

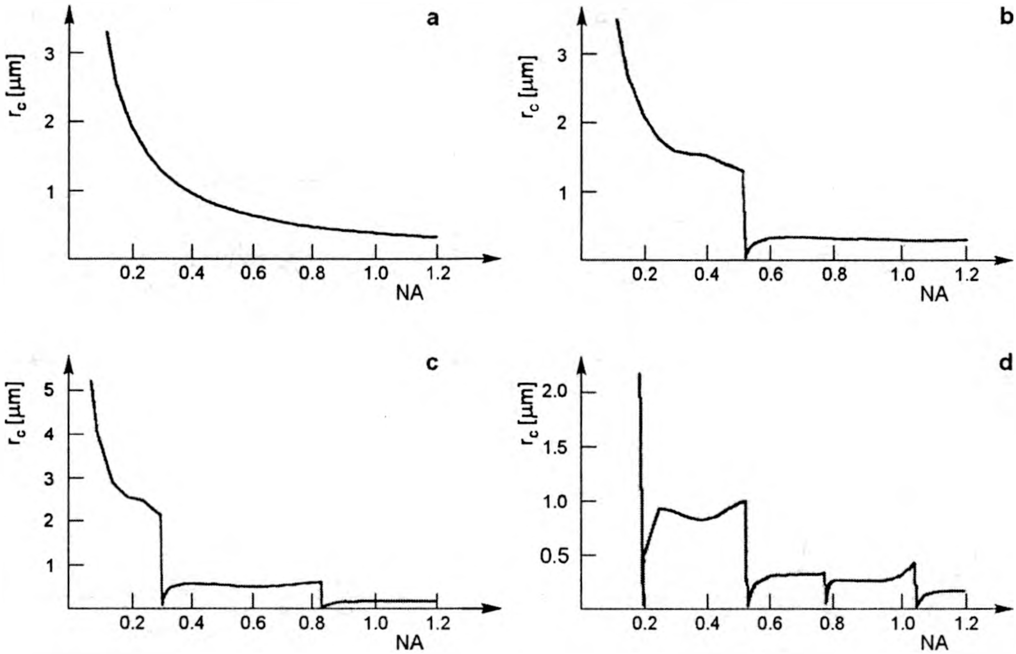

Fig. 2. Cut-off spatial frequencies r_c for different values of the numerical aperture NA = 0.05, 0.1,...,1.2 for the pupil filter of $\cos(N\rho)$ type for the following parameters: N = 1 (**a**), N = 4.5 (**b**), N = 7.5 (**c**), N = 12.0 (**d**).

takes the minimum value $r_c = 0.0495$ μm for NA = 0.311 and $r_c = 0.0113$ μm for NA = 0.8379 (Fig. 2**c**). For $N = 12.0$ the cut-off frequency takes the following minimum values: $r_c = 0.0407$ μm for NA = 0.1942, $r_c = 0.0385$ μm for NA = 0.524, $r_c = 0.0694$ μm for NA = 0.768 and $r_c = 0.0382$ μm for NA = 1.047 (Fig 2**d**).

For CSM with the aperture modulated by the filters of $\cos^2(N\rho)$ type for $N = 1$ the cut-off frequency changes from the value $r_c = 7.7207$ μm for NA = 0.05 to the value $r_c = 0.4367$ μm for NA = 1.2 (see Fig. 3**a**). For the parameter $N = 2.5$ the cut-off frequency changes its NA = 1.025 (see Fig. 3**b**). For $N = 5.0$, r_c changes significantly for NA = 0.52 (see Fig. 3**c**). For $N = 7.0$, r_c changes its value for NA = 0.37 (see Fig. 3**d**). For $N = 12.0$, r_c changes significantly its value for NA = 0.22 (see Fig. 3**e**) and for $N = 15.0$ the cut-off frequency varies from $r_c = 8.45$ μm for NA = 0.05 to $r_c = 0.3346$ μm for NA = 1.15 (see Fig. 3**f**). Thus for CSM modulated with the filters of $\cos(N\rho)$ type for the numerical aperture NA = 0.2 we obtain the cut-off frequency $r_c = 1.935$ μm for $\cos(\rho)$, $r_c = 2.063$ μm for $\cos(4.5\rho)$, $r_c = 2.566$ μm for $\cos(7.5\rho)$, $r_c = 0.517$ μm for $\cos(12\rho)$ and for nonmodulated CSM ($N = 0$) for the numerical aperture NA = 0.2, $r_c = 1.93$ μm. For the numerical aperture NA = 0.4 we obtain $r_c = 0.976$ μm for $\cos(\rho)$, $r_c = 1.517$ μm for $\cos(4.5\rho)$, $r_c = 0.59$ μm for $\cos(7.5\rho)$, $r_c = 0.839$ μm for $\cos(12\rho)$ while for $N = 0$ (nonmodulated CSM) for NA = 0.4 we get

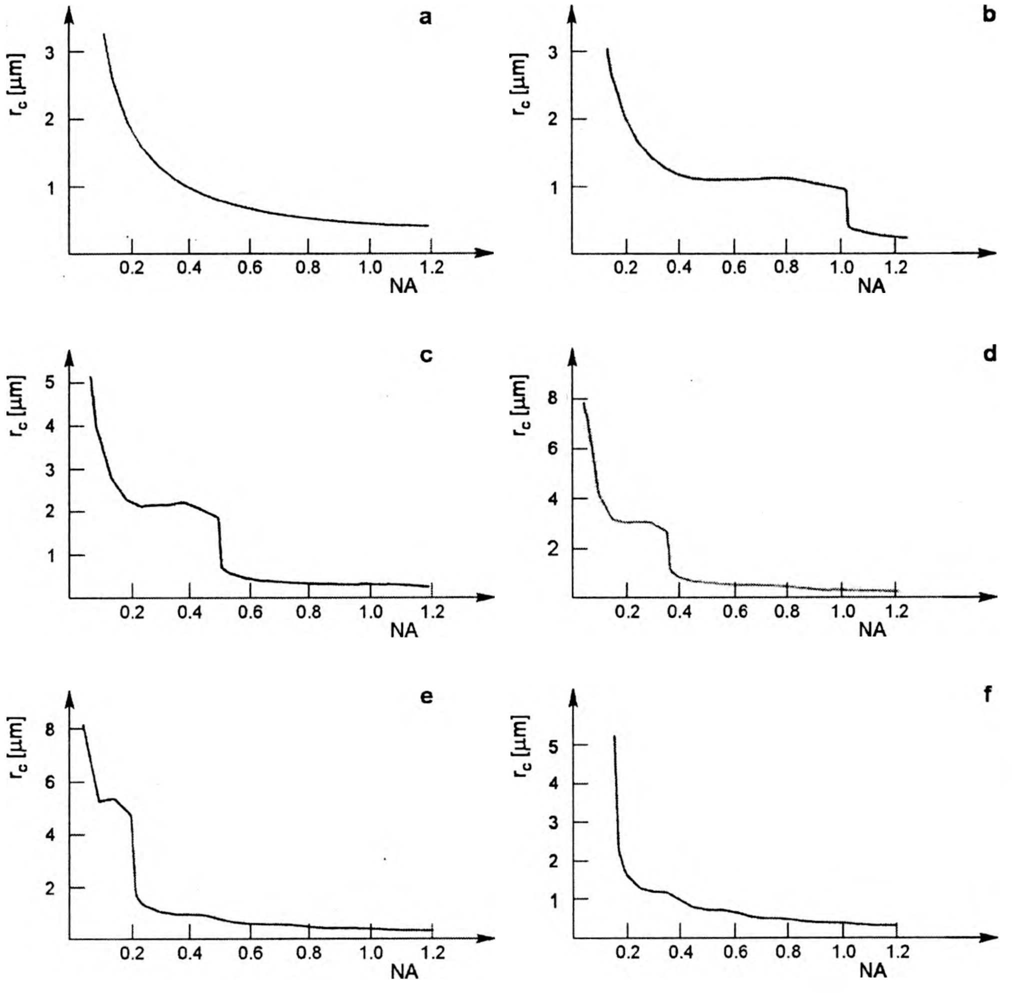

Fig. 3. Cut-off spatial frequencies r_c for different values of the numerical aperture NA = 0.05, 0.1, ...,1.0 for the pupil filter of $\cos^2(N\rho)$ type for the following parameters: $N = 1$ (**a**), $N = 2.5$ (**b**), $N = 5.0$ (**c**), $N = 7.0$ (**d**), $N = 12.0$ (**e**), $N = 15.0$ (**f**).

$r_c = 0.965$ μm. For numerical aperture NA = 1.0 the following estimations have been made: $r_c = 0.4211$ μm for $\cos(\rho)$, $r_c = 0.3157$ μm for $\cos(4.5\rho)$, $r_c = 0.209$ μm for $\cos(7.5\rho)$, $r_c = 0.323$ μm for $\cos(12\rho)$ and for $N = 0$ (NA = 1.0) $r_c = 0.386$ μm.

For CSM of the aperture modulated by the filters of $\cos^2(N\rho)$ type for numerical aperture NA = 0.2 the corresponding data are: $r_c = 1.94$ μm for $\cos^2(\rho)$, $r_c = 2.002$ μm for $\cos^2(2.5\rho)$, $r_c = 2.323$ μm for $\cos^2(5\rho)$, $r_c = 3.06$ μm for $\cos^2(7\rho)$, $r_c = 4.836$ μm for $\cos^2(12\rho)$, $r_c = 1.635$ μm for $\cos^2(15\rho)$ while for $N = 0$, NA = 0.2, $r_c = 1.93$ μm. For the numerical aperture NA = 0.4, $r_c = 0.987$ μm for $\cos^2(\rho)$, $r_c = 1.162$ μm for $\cos^2(2.5\rho)$, $r_c = 2.25$ μm for $\cos^2(5\rho)$, $r_c = 0.866$ μm for $\cos^2(7\rho)$, $r_c = 0.9662$ μm for $\cos^2(12\rho)$, $r_c = 0.9897$ μm for $\cos^2(15\rho)$. On the other hand for $N = 0$, NA = 0.4,

$r_c = 0.965$ μm. For NA = 1.0, we obtain $r_c = 0.465$ μm for $\cos^2(\rho)$, $r_c = 0.9758$ μm for $\cos^2(2.5\rho)$, $r_c = 0.4015$ μm for $\cos^2(5\rho)$, $r_c = 0.3557$ μm for $\cos^2(7\rho)$, for $r_c = 0.3996$ μm for $\cos^2(12\rho)$, while for $N = 0$, NA = 1.0, $r_c = 0.386$ μm. In paper [3], it has been shown how the trigonometric filters influence the axial and transverse gains. In paper [4], it has been shown how the phase pupil filters improve the longitudinal resolution in a confocal scanning microscope.

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