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Letters to the Editor

Remarks on effects of aberrating layers in confocal scanning microscopes

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In paper [1], the condition for aberration-free immersion layer in aberration-free confocal scanning microscope (CSM) is given as dependent on λ , α , n_1 , n_2 . A spherical aberration coefficient of the first order for the layer is equal to [1]

$$W_{40} = 2kt(n_2^2 - n_1^2) \frac{n_1^2}{n_2^3} \sin^4(\alpha/2).$$
⁽¹⁾

where: α - semi-angle of convergence, and $k = 2\pi/\lambda$, while n_1 , n_2 , t and θ are defined in Fig. 1.

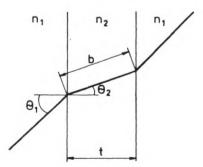


Fig. 1. Ray incident on a dielectric slab

Assuming the Rayleigh criterion, according to which the maximum of phase aberration must be less than $\pi/2$ which corresponds to the limiting resolution $\lambda/4$, the condition for aberration-free layer thickness t has been obtained [1]

$$t \leq \lambda n_2^3 / \{2n_1^2(n_2^2 - n_1^2)\sin^4(\alpha/2)\}$$
(2)

In this paper, a correcting term to the condition (2) has been determined as related to spherical aberration β_{040} of CSM depending on Δ_{limCSM} , k, α, n_1, n_2 ; where Δ_{limCMS} – limiting resolution of CSM, $n_2 = n_1 + \Delta n$. A correcting coefficient $W = \frac{\Delta_{\text{max}}}{\Delta_{\text{min}}}$ for an apodized CSM system suffering from spherical aberration has been

introduced, where Δ_{\min} — minimum value of limiting resolution in CSM with apodization and spherical aberration, Δ_{\max} — limiting resolution in CSM of uniform type. In further considerations, it has been assumed that the refraction index n_2 differs only slightly from n_1 . The intensity distribution in the focal plane of CSM is defined by the relation [1]

$$I(u,v) = \left| \int_{0}^{u} A(\theta) P(\theta) J_{0}\left(\frac{v\sin\theta}{\sin\alpha}\right) \exp\left(-\frac{1}{2}iu \frac{\sin^{2}(\theta/2)}{\sin^{2}(\alpha/2)}\right) \sin\theta d\theta \right|^{2}$$
(3)

where u, v — optical coordinates which are defined by the axial distance z from the focus and radial distance r from the optical axis in the following way: $u = 4kz\sin^2(\alpha/2), v = kr\sin\alpha, A(\theta_1)$ for aplanatic system is equal to $A(\theta_1) = \cos^{1/2}\theta_1$, $P(\theta_1)$ — wavefront aberration, $P(\theta_1) = e^{i\varphi}$. Basing on the formula (3), the limiting resolution of CSM denoted by Δ_{limCSM} has been numerically evaluated. The total spherical wave aberration of first order for the combination CSM plus immersion layer fullfils the condition

$$\Phi_{\max} = \frac{kt}{2} (n_2^2 - n_1^2) \frac{n_1^2}{n_2^3} \sin^4(\alpha/2) + \Delta_{\lim} = 0,$$

$$\Delta_{\lim} = \Delta_{\lim} CSM.$$

Hence

$$t \leq 2\Delta_{\lim} n_2^3 / \{k(n_2^2 - n_1^2) n_1^2 \sin^4(\alpha/2)\}.$$
(4)

For $n_2 = n_1 + \Delta n$ we have

$$\frac{(n_1+\Delta n)^3}{(2n_1\Delta n+\Delta n^2)n_1^2}\simeq\frac{n_1+3\Delta n}{2n_1\Delta n},$$

and, consequently,

$$t \leq \left[2\Delta_{\lim}/k\sin^4(\alpha/2)\right] \left[\frac{n_1 + 3\Delta n}{2n_1\Delta n}\right] \simeq \frac{2W(n_1 + 3\Delta n)}{2n_1\Delta nk\sin^4(\alpha/2)},\tag{5}$$

The limiting value of resolution in a nonapodized and aberration-free CSM amounts to $\Delta_{\text{lim}} = 2.89$, which was shown in paper [2]. In CSM equipped with an apodized collector and objective with the apodizer of r^2 type, the limiting value of Δ_{lim} is equal to 1.31. For CMS charged with spherical aberration $\beta_{040} = 0.5 - 1.5$ the limiting value Δ_{lim} does not exceed Δ_{limCSM} for the uniform case (Tab. 1). With the increase of α from 0.1 to 1.6, $\Delta_{\text{lim}}/\sin^4(\alpha/2)$ diminishes (Fig. 2). The correcting coefficient $W = \frac{\Delta_{\text{max}}}{\Delta_{\text{min}}}$ has been calculated again from formula (3) (Tab. 2) for the respective two cases.

For the classic optical system, for which $\Delta = 3.83$ the correcting coefficient W is equal to ~2.9. For the sake of comparison, the same intervals for refractive index were assumed as those used in paper [1]. In CSM with $\lambda = 633$ nm, two cases were

A.	A,	Δ_{lim}	a	$\Delta_{\rm lim}/\sin^4(\alpha/2)$
Uniform	Uniform	2.976	0.1	476954
,2	r ²	1.31		209950
s = 0	$\varepsilon = 0.25$	1.48		237195
s = 0.5	$\varepsilon = 0.5$	1.425		228380
$\varepsilon = 0.9$	$\varepsilon = 0.9$	1.40		224374
$\beta_{040}=0$	$\beta_{040}=0$	2.98		477595
$\beta_{040} = 0.5$	$\beta_{040} = 0.5$	2.96		474390
$\beta_{040}=1$	$\beta_{040} = 1$	2.98		477595
$\beta_{040} = 1.5$	$\beta_{040}=1.5$	2.87		459966
Uniform	Uniform	2.976	0.5	794.34
r ²	r ²	1.31		349.659
ε = 0	$\varepsilon = 0.25$	1.48		395.035
$\varepsilon = 0.5$	$\varepsilon = 0.5$	1.425		380.35
$\varepsilon = 0.9$	$\varepsilon = 0.9$	1.40		373.681
$\beta_{040}=0$	$\beta_{040}=0$	2.98		795.408
$\beta_{040}=0$	$\beta_{040} = 1.5$	2.88		768.716
$\beta_{040}=0.5$	$\beta_{040} = 0.5$	2.96		790.069
$\beta_{040} = 1.5$	$\beta_{040} = 1.5$	2.87		766.047
Uniform	Uniform	2.976	1	56.331
r ²	r ²	1.31		24.7963
ε = 0	$\varepsilon = 0.25$	1.48		28.014
e = 0.5	$\varepsilon = 0.5$	1.425		26.973
e = 0.9	$\varepsilon = 0.9$	1.40		26.499
$\beta_{040} = 0$	$\beta_{040}=0$	2.98		56.407
$\beta_{040} = 0$	$\beta_{040} = 1.5$	2.88		54.514
$\beta_{040} = 0.5$	$\beta_{040}=0.5$	2.96		56.028
$\beta_{040} = 1.5$	$\beta_{040}=1.5$	2.87		54.325
Uniform	Uniform	2.976	1.5	13.785
r ²	r ²	1.31		6.028
$\varepsilon = 0$	$\varepsilon = 0.25$	1.48		6.856
s = 0.5	$\varepsilon = 0.5$	1.425		6.60
ε = 0.9	$\varepsilon = 0.9$	1.40		6.48
$\beta_{040}=0$	$\beta_{040} = 0$	2.98		13.804
$\beta_{040}=0$	$\beta_{040} = 1.5$	2.88		13.3406
$\beta_{040} = 0.5$	$\beta_{040} = 0.5$	2.96		13.7116
$\beta_{040} = 1.5$	$\beta_{040} = 1.5$	2.87		13.2943

Table 1. Dependence of the limiting resolution Δ_{llm} on α in CSM with apodization of r^2 type, annular ε and spherical aberration β_{040} (A_{ε} – objective, A_{ε} collector, ε – circular central obstruction)

Table 2. Aberration correcting coefficient in CSM with apodization optimal in uniform CSM (Δ_{mim} – limiting resolution in CSM with r^2 apodization, Δ_{max} – limiting resolution in uniform CSM)

α		$\Delta_{\rm lim}/\sin^4(\alpha/2)$	$W = \Delta_{\max} / \Delta_{\min}$
1	2	3	4
0.1	$\Delta_{min} = 1.31$	209950	~2.27
	$\Delta_{\rm min} = 1.31$ $\Delta_{\rm max} = 2.98$	477595	

1	2	3	4
0.5	$\Delta_{\min} = 1.31$	349.659	~2.27
	$\Delta_{\rm max} = 2.98$	795.408	
1.5	$\Delta_{\rm min} = 1.31$	6.068	~2.27
	$\Delta_{\rm max} = 2.98$	13.804	
1.6	$\Delta_{min} = 1.31$	4.947	~2.27
	$\Delta_{\rm max} = 2.98$	11.2532	

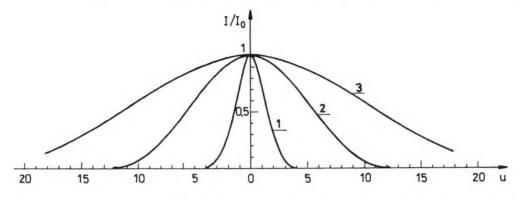


Fig. 2. Intensity in focal region in CSM as dependent on α (semi-angle of convergence), curve $1 - \alpha = 0.5$, curve $2 - \alpha = 1$, curve $3 - \alpha = 1.5$

calculated. Case 1: $\Delta n = 0.01$ (while n_2 ranging within the interval 1.513-1.523), $n_1 = 1$, $t_{optCSM} = 19.32 \ \mu m$. Case 2: $\Delta n = 0.033$ (while n_2 ranging within the interval 1.514-1.481), $n_1 = 1$, $t_{optCSM} \simeq 6.25 \ \mu m$. Optimal thickness of the immersion layer should be adjusted to the resolution of CSM which in the first case corresponds to the value 19.32 μm , while in the second case to the value 6.25 μm .

References

[1] SHEPPARD C. J. R., COGSWELL C. J., Optik 87 (1991), 34.

[2] MAGIERA A., Atti Fond. Giorgio Ronchi 45 (1990), 873.

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