Improvement of three-dimensional resolution in confocal scanning microscopy by combination of two pupil filters

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Improvement of three-dimensional resolution in confocal scanning microscopy by combination of two pupil filters. A simple technique for improving 3-D resolution in confocal scanning microscopy is presented. The technique is based on the equal contribution to the image of the illuminating and the collecting lenses. It is proposed, then, to apodize such lenses with complementary filters, the one designed for increasing the resolution in the image plane, and the other for achieving axial superresolution. Each pupil independently introduces zeros in the point spread function, the positions of which can be chosen to lie in the focal plane or along the optic axis. The combined action of both filters produces a narrowing of the point spread function of the system both in the image plane, and along the optical axis. A numerical example with two well-known pupil filters is carried out.

Verbesserung der dreidimensionalen Auflösung im konfokalen Rastermikroskop durch Kombination zweier Pupillenfilter. Es wird eine einfache Methode zur Verbesserung der dreidimensionalen Auflösung im konfokalen Rastermikroskop vorgestellt. Die Methode basiert auf der entsprechenden Verteilung der Bilder in der Beleuchtungs- und Sammellinse. Es wird vorgeschlagen, diese Linsen mit komplementären Filtern zu apodisieren, das eine zur Erhöhung der Auflösung in der Bildebene und das andere zur Erreichung von axialer Superauflösung. Jede Pupille führt unabhängig Nullstellen in der Punktbild-Übertragungsfunktion ein, deren Position in der Fokusebene oder entlang der optischen Achse gewählt werden kann. Die gemeinsame Antwort beider Filter gibt eine Verschmälerung in der Punktbild-Übertragungsfunktion des Systems in der Bildebene wie auch entlang der optischen Achse. Ein numerisches Beispiel mit zwei bekannten Pupillen-Filtern wird vorgestellt.

1 Introduction

Confocal scanning microscopes (CSM) are imaging systems in which the light radiated from a point source is focused onto the object by an illuminating set, then the transmitted light is imaged by a collecting set and the light which passes through a pinhole at the center of the image plane is detected. In this symmetrical configuration both the illuminating and collecting sets, play equal roles in the image properties. The most important feature of the CSM is their ability to form a three-dimensional (3-D) image of 3-D objects [1]. This 3-D capability results from both their high transverse resolution capacity and their optical sectioning ability. Over the last years several attempts have been made in order to achieve an improvement of the resolution capacity of the CSM either in the transverse direction [2–6], or along the axial direction [7–9].

The goal of this work is to report a technique for improving the resolution capacity of the CSM simultaneously in the transverse and the axial direction. The technique is based on a proper combination of two different pupil filters, the one designed for increasing the resolution in the image plane, and the other for achieving axial superresolution. It will be shown that with this combination it is possible to reduce globally the size of the central lobe of the 3-D point spread function (PSF) of the CSM. This important property results from the fact that each of the pupils gives rise to zeros in intensity in the final effective PSF. Previously Hegedus [2] has shown that the zeros can be chosen to lie in the focal plane. Here we demonstrate that the zeros can lie both on the optic axis and in the focal plane, thus resulting in improved 3-D imaging.

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2 Basic theory

For our present discussion we start by considering a transmission CSM in which the pupils of illuminating and collecting sets are apodized by the radially-symmetric pupil filters of amplitude transmittance $\tilde{p}_1(\rho)$ and $\tilde{p}_2(\rho)$, respectively (see fig. 1). Then the intensity distribution in the image of an axial point source, i.e. the 3-D intensity PSF, is given by [10]

$$I(v, W_{20}) = |p_1(v, W_{20})\, p_2(v, W_{20})|^2\,, \tag{1}$$

being

$$p(v, W_{20}) = 2\pi \int_0^1 \tilde{p}(\rho) \exp(-i\, 2\pi\, W_{20}\, \rho^2)\, J_o(2\pi\, v\, \rho)\, \rho\, d\rho\,, \tag{2}$$

where a unit-radius pupil is assumed.

In eq. (2) v is a normalized coordinate related to the radial coordinate, r, by $v = r_o\, r/\lambda f$, where r_o is the maximum radial extension of the pupil, and f the focal length

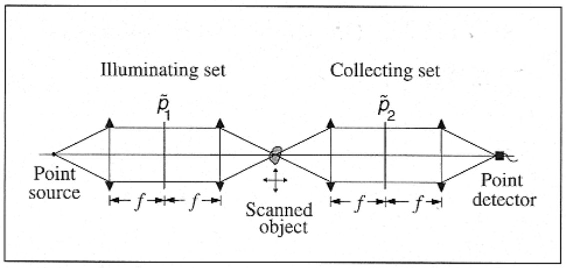

Fig. 1. Scheme of the transmission confocal scanning system.

of the system. The coefficient W_{20} is the well-known defocus coefficient that specifies the amount of defocus measured in units of wavelength.

From eqs. (1) and (2), it is clear that the 3-D intensity PSF of a confocally scanned system is governed by both the amplitude transmittance of the illuminating-set pupil and that of the collecting-set pupil. This particular property can be exploited for shaping the 3-D PSF by mean of a combination of two different nonuniform transmission pupil filters.

Since we are interested in increasing the resolution capacity of a confocally scanned system, a pair of filters should be selected such that the width of the central lobe of the 3-D PSF of the CSM is reduced both in transverse and in axial directions. To reach this end, we follow the next reasoning.

First we particularize eq. (2) for the two cases of our interest: the amplitude distribution in the image plane, and that along the optical axis. For the focal plane, $W_{20} = 0$, we have that

$$p(v, 0) = 2\pi \int_0^1 \tilde{p}(\rho) J_o(2\pi v \rho) \rho \, d\rho . \quad (3)$$

Then the two-dimensional (2-D) amplitude distribution in the image plane, what we will refer to hereafter as the transverse amplitude PSF, is given by the Hankel transform of the amplitude transmittance, $\tilde{p}(\rho)$, of the filter. So, if we want to produce an effect of transverse superresolution, a filter that enhances the high frequencies over the low frequencies is necessary, that is, a purely absorbing pupil filter whose maximum transmittance is at $\rho = 1$ and whose minimum transmittance is at $\rho = 0$.

On the other hand, for the case of the optical axis, $v = 0$, we have that

$$p(0, W_{20}) = 2\pi \int_0^1 \tilde{p}(\rho) \exp(-i 2\pi W_{20} \rho^2) \rho \, d\rho . \quad (4)$$

This equation can be converted into a one-dimensional (1-D) Fourier transform by using the next geometrical mapping,

$$\zeta = \rho^2 - 0.5 , \qquad q(\zeta) = \tilde{p}(\rho) . \quad (5)$$

Under this variable change, eq. (4) can be rewritten, aside from irrelevant premultiplying phase and constant factors, as

$$p(0, W_{20}) = \int_{-0.5}^{0.5} q(\zeta) \exp(-i 2\pi W_{20} \zeta) \, d\zeta . \quad (6)$$

Then, the axial amplitude distribution is given by the 1-D Fourier transform of the function $q(\zeta)$. In order to investigate which characteristics a filter should have for producing an axial superresolving effect, it is convenient to point out the similarity existing between this situation and that corresponding to the analysis of a 1-D imaging formation system. For the latter case, the transmittance of a 1-D pupil filter and its corresponding 1-D amplitude PSF are also connected by an 1-D Fourier transformation.

From the above analogy one can infer, as is made clear by Ojeda-Castañeda et al. [11], that for obtaining axial superresolution a filter is necessary whose mapped function $q(\zeta)$ enhances the "high frequencies" over the "low frequencies", in other words, it is convenient that the amplitude transmittance of the purely absorbing filter, $\tilde{p}(\rho)$, is maximum at $\rho = 0$ and at $\rho = 1$, and minimum at $\rho = \sqrt{2}/2$.

3 Combination of two complementary filters

It is clear that a pupil filter designed for increasing the transverse resolution capacity of an optical system, produces a narrowing of the central lobe of its transverse PSF, which is accompanied by an increase in the strength of secondary side lobes, and a widening of the central lobe of its axial PSF. On the contrary, if the filter is designed for achieving axial superresolution, an increase in the width of the central lobe of the transverse PSF appears, in general, as a collateral effect [12].

As has been established in eq. (1), the 3-D intensity PSF of a CSM is given by the product of two independent 3-D PSFs, one corresponding to the illuminating set and the other to the collecting set. The independence of these two factors permits us to select a couple of filters which have the ability of producing the wanted complementary effects. In this way, for reaching the aim of reducing the area of the central lobe of 3-D PSF of the CSM, we propose to combine a filter designed for producing transverse superresolution, with an axially superresolving filter.

When the product of the two independent 3-D PSFs is performed, a 3-D amplitude distribution is obtained in which the width of the central lobe in the transverse direction is governed by the PSF of the transverse superresolving filter, whereas the width in the axial direction is imposed by the PSF corresponding to the axially superresolving filter. A similar reasoning can be applied to any other direction passing through the focus. In this general case the width of the central lobe in any direction is imposed by the narrowest of the independent PSF in such direction. In this way, a 3-D PSF can be obtained whose central lobe dimensions are globally reduced in comparison with those corresponding to a nonapodized CSM. Thus, it can be stated that the proper combination of two pupil filters provides a 3-D superresolving effect.

At this point we would like to emphasize that the use of purely absorbing pupil filters of continuously varying amplitude transmittance does not constitute a technolog-

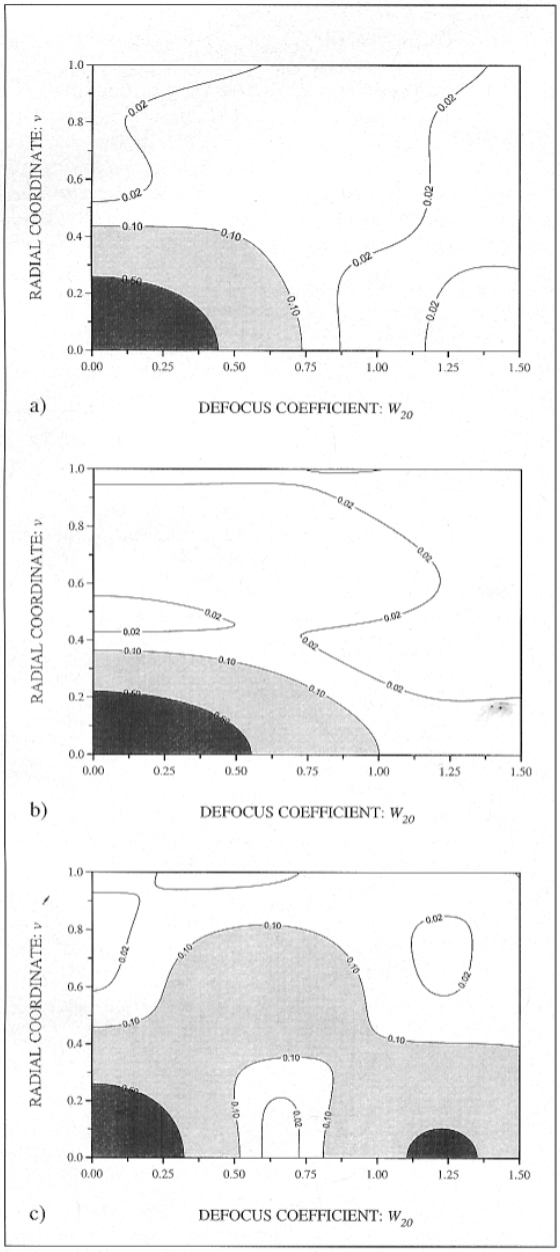

Fig. 2. **Numerically evaluated contours of equal normalized intensity corresponding to the 3-D PSF of: a) circular aperture; b) transverse superresolving parabolic filter, $\tilde{p}(\rho) = \rho^2$; c) axially superresolving parabolic filter, $\tilde{p}(\rho) = 4\rho^4 - 4\rho^2 + 1$.**

ical problem. As has been stated by some authors [2, 13, 14], the use of an adequate digital halftoning technique permits the manufacture of binary masks such that the difference between their PSF and that of the continuous pupil filter is negligible.

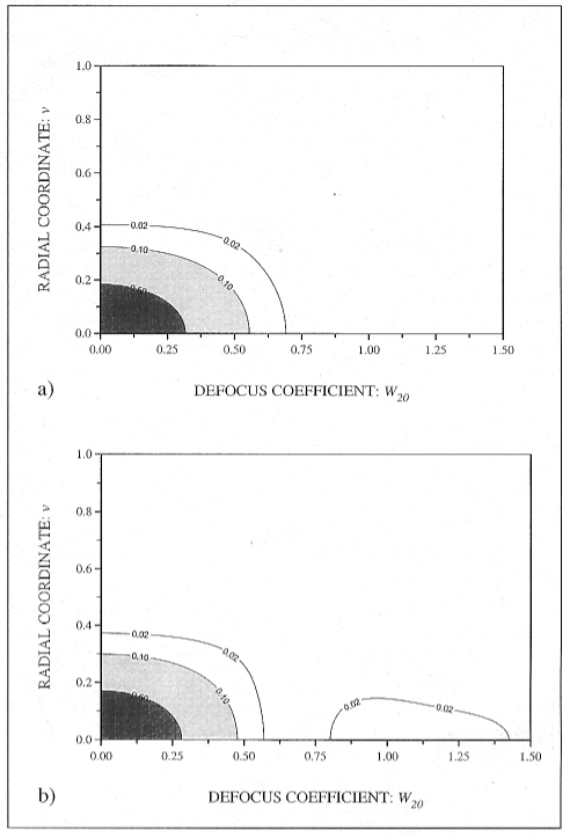

Fig. 3. **Theoretical contours of equal normalized intensity corresponding to the 3-D PSF of: a) a nonapodized CSM; b) a CSM in which the illuminating and collecting sets are apodized by the filters $\tilde{p}_1(\rho) = \rho^2$ and $\tilde{p}_2(\rho) = 4\rho^4 - 4\rho^2 + 1$.**

4 Numerical example

In order to illustrate our result we select as a couple of filters the well known transverse superresolving parabolic filter $\tilde{p}_1(\rho) = \rho^2$, and the axially superresolving parabolic filter $q_2(\zeta) = 4\zeta^2$, whose representation in radial coodinates in $\tilde{p}_2(\rho) = 4\rho^4 - 4\rho^2 + 1$. In fig. 2 we have represented, in isophotes diagrams, the normalized 3-D intensity PSFs corresponding to the selected filters, beside that corresponding to the nonapodized circular aperture. Note, from these plots, that the narrowing of the central lobe in the selected direction is accompanied by an increase in the strength of the secondary lobes, and by a widening in the opposite direction.

Next, in fig. 3b we have depicted the normalized 3-D intensity PSF of a CSM in which the collecting-set pupil is apodized by one of the above proposed filters and the illuminating-set pupil by the other, assuming that both pupil-filters have the same external radius. When comparing this figure with figs. 2b–c, we recognize that the width of the central lobe in transverse direction is imposed, precisely, by the transverse superresolving filter, and that the width in the axial direction is governed by the axially superresolving filter. Moreover, the height of the secondary lobes at any direction, passing through the focus, has been drastically reduced in comparison with the corresponding to the independent 3-D PSF. If we compare now this intensity distribution with that corresponding to a nonapodized CSM (fig. 3a), it is clear that there is an important reduction of the width of the central lobe at any direction, and hence in its global volume. In particular, the width of the central lobe exhibits a reduction of 21% in the transverse direction and of 34% along the axis. In terms of the half-intensity, the percentage reduction in the transverse and axial directions are 7.3% and 11%, respectively.

The proposed method can be used in either the reflection or the transmission geometry, and in either bright-field or fluorescence imaging modes. For the latter in particular the signal collection efficiency is of great importance. Thus as the filter $\tilde{p}_1(\rho)$ gives a Strehl ratio of 1/4, whereas that for $\tilde{p}_2(\rho)$ is 1/9, it is preferable to use $\tilde{p}_1(\rho)$ in the collecting set.

In reflection imaging, the image of a planar object is of particular importance. The axial image amplitude from a level planar object is given by the 2-D Fourier transform of the product of the mapped pupils. For the example given here, the axial image from a plane is broader than for the system without filters. However it should be stressed that there is considerable flexibility in overall design of the system, and that the product of the pupils can be designed accordingly.

5 Conclusions

We have proposed a novel technique for improving the 3-D resolution capacity of a CSM. The cornerstone of our approach is to combine the effects of two properly selected pupil filters. We have shown that by this quite simple method it is possible to achieve a high reduction of the width of the central lobe of the 3-D PSF both in the transverse and in the axial directions. To illustrate our result, we have performed a numerical example in which we have evaluated the 3-D intensity PSF of a CSM apodized by two well-known pupil filters. This numerical example allowed us to recognize an important reduction of the size of the central lobe of PSF in terms both of the zero and the half-intensity width.

Previously, it has been shown that improving transverse resolution usually results in degradation of the imaging performance in the axial direction [12]. Thus improving resolution in both transverse and axial directions is an important advance. The method is based on the independent specification of zeros in the effective point spread function both in the focal plane and along the optic axis. This basic approach is applicable to more complex filters, which have the potential to achieve better performance than the very simple filters considered here.

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