Improvement of three-dimensional resolution in confocal microscopy

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ABSTRACT

It is presented a technique for improving 3-D resolution in confocal scanning microscopy. 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 combined action of both filters produces a narrowness of the point spread function of the system both in the image plane, and along the optical axis.

1. INTRODUCTION

The intensity distribution in the three-dimensional (3-D) image of a point source provided by an imaging system, i.e., the intensity point spread function (PSF) of the system, is governed by the finite extent and the amplitude transmission of the exit pupil as well as the wavelength of the radiation. Several efforts have been addressed to modify the characteristics of this PSF in order to improve the quality of the image. In this sense, the use of nonuniform transmission filters to produce apodization[1,2] or superresolution[1-3] on the transverse intensity PSF, i.e., on the intensity distribution at the image plane, is well known.

More recently, the effects of nonuniform transmission filters in the axial PSF have been studied. In this sense, certain filters have been proposed for achieving high focal depth,[4,5] for reducing the influence of spherical aberrations,[6] for obtaining zero-axial irradiance for optical alignment,[7] or even for removing the energy from the axial point of the image plane with the aim of achieving high precision focusing.[8] The design of filters for achieving superresolution along the optical axis is of great importance in 3-D imaging because the narrower is the central lobe of the axial response, the higher is the optical-sectioning capacity of the system. However, not too much attention has been paid to the design of this kind of filters.[9,10]

On the other hand, 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 3-D image of a 3-D object.[11] This 3-D capability results from both their high transverse resolution capacity and their optical sectioning ability. Along 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,[12-16] or along the axial direction.[17,18]

The goal of this work is to report a new 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, one designed for increasing the resolution in the image plane, an the other for achieving axial superresolution. It will be shown that the combined action of both filters produces a narrowness of the point spread function of the system both in the image plane, and along the optical axis.

2. BASIC THEORY

For our present discussion we start by considering a CSM in which the pupils of illuminating and collecting sets are apodized by the radially-symmetric functions by $\tilde{p}_1(\rho)$ and $\tilde{p}_2(\rho)$, respectively (see Fig.1). Then the intensity distribution in the image of a point source, i.e. the intensity PSF, is given by[19]

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

where

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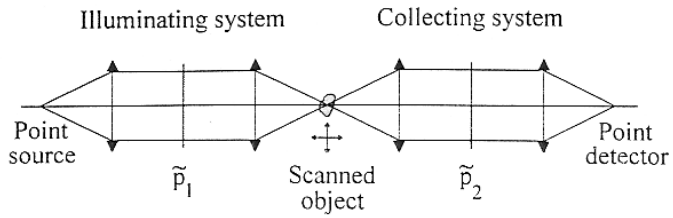

Fig.1. Scheme of a confocal imaging system.

$$p(v, W_{20}) = 2\pi \int_0^1 \tilde{p}(\rho)\, exp(-i2\pi W_{20}\rho^2)\, J_o(2\pi v\rho)\, \rho\, d\rho \quad , \tag{2}$$

where the unit-radius pupil is assumed.

In Eq.(2) v is a normalized coordinate related to the radial coordinate by $v = r_o r/\lambda f$, being r_o the maximum radial extension of the pupil and f the focal length of the system. Coefficient W_{20} specifies the amount of defocus measured in units of wavelength.

From Eqs.(1) and (2), it is clear that the 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 PSF by mean of a combination of two different pupil functions.

Since we are interested in increasing the resolution capacity of a confocally scanned system, it should be selected a pair of filters such that the width of the central lobe of the PSF of the CSM is reduced both in transverse and in axial direction. 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

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

then the two-dimensional (2-D) amplitude distribution in the image plane, what we will refer hereafter as the transverse amplitude PSF, is given by the Hankel transformation of the amplitude transmittance, $\tilde{p}(\rho)$, of the filter. So, if we want to produce an effect of transverse superresolution, it is necessary a filter that enhances the high frequencies over the low frequencies, that is, a 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 we have that

$$p(0, W_{20}) = 2\pi \int_0^1 \tilde{p}(\rho)\, exp(-i2\pi W_{20}\rho^2)\, \rho\, d\rho \quad . \tag{4}$$

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

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

Under this variable change, Eq.(4) can be rewritten, aside for irrelevant phase and constant factors, as

$$p(0, W_{20}) = \int_{-0.5}^{0.5} q(\zeta)\, exp(-i2\pi W_{20}\zeta)\, d\zeta \quad . \tag{6}$$

Then, the axial amplitude distribution is given by the 1-D Fourier transform of the function $q(\zeta)$. In order to investigate which characteristics should have a filter for producing an axial superresolving effect, it is convenient to point out the similarity existing between this situation and the corresponding to the analysis of the 1-D imaging formation systems. In this 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 it is made in reference 20, that for obtaining axial superresolution it is necessary a filter 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 filter, $\tilde{p}(\rho)$, is maximum at $\rho = 0$ and at $\rho = 1$, and minimum at $\rho = \sqrt{2}/2$.

3. COMBINATION OF COMPLEMENTARY FILTERS

It is clear that a pupil filter designed for increasing the transverse resolution capacity of an optical system, produces a narrowness of the central lobe of its transverse PSF, which is accompanied by an increasing of 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 increasing of the width of the central lobe of transverse PSF appears, in general, as a collateral effect.[10]

As it has been established in Eq.(1), the intensity PSF of a CSM is given by the product of two independent PSF, the corresponding to the illuminating set and that of the collecting set. The independence of these two factors permits to select a couple of filters which have the ability of producing complementary effects. In this way, for reach 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 PSF is performed, it is obtained an amplitude distribution in which the width of the central lobe in the transverse direction is the same than that of 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 at any direction is imposed by the narrowest of the independent PSF in such direction. In this way, it can be obtained a PSF whose central lobe is globally reduced in comparison with that corresponding to a nonapodized CSM. Thus, it can be stated that the proper combination of two pupil filters provides an 3-D superresolving effect.

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 coordinates is $\tilde{p}_2(\rho) = 4\rho^4 - 4\rho^2 + 1$. In Fig.2 we have represented the normalized intensity PSF, in terms of both axial and radial coordinate, corresponding to the selected filters, and that of the nonapodized circular aperture. Note, from this figure, that the narrowness of the central lobe in the selected direction is accompanied by an increasing of the strength of the secondary lobes, and by a widening in the opposite direction.

Next, in Fig.3 we have depicted, with dashed lines, the normalized 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, supposed to have both pupil-filters the same external radius. When comparing this figure with Fig.2, 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. If we compare now this intensity distribution with the corresponding to the nonapodized CSM (solid line in Fig.3), it is clear that there is an important reduction of the width of the central lobe at any direction. In particular, the width of the central lobe suffers a reduction of a 21% in transverse direction and of a 34% in the axis.

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 it is possible to achieve a high reduction of the width of the central lobe of the PSF both in transverse and in axial direction. To illustrate our result, 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 intensity PSF.

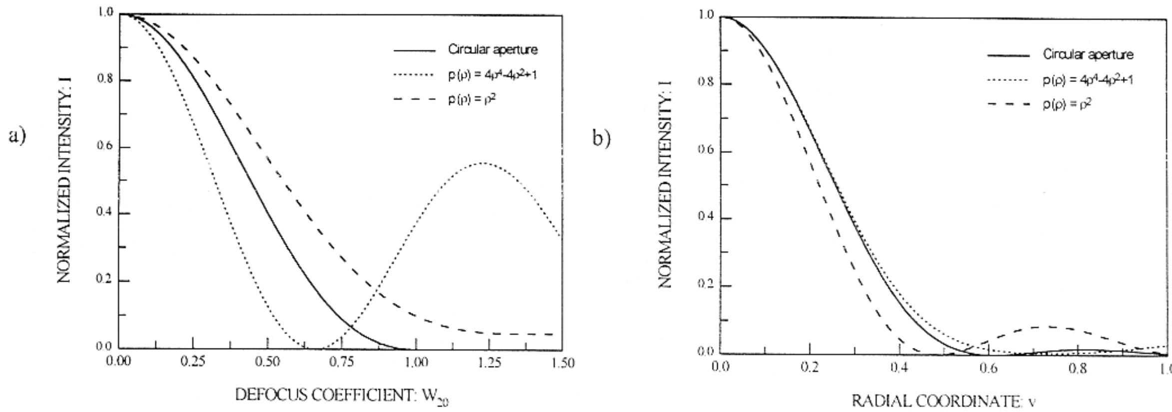

Fig.2. a) Normalized axial intensity PSF of: circular aperture (solid line), transverse superresolving parabolic filter, $\tilde{p}(\rho) = \rho^2$ (dashed line), axially superresolving parabolic filter, $\tilde{p}(\rho) = 4\rho^4 - 4\rho^2 + 1$ (dotted line); b) Normalized transverse intensity PSF for the same cases.

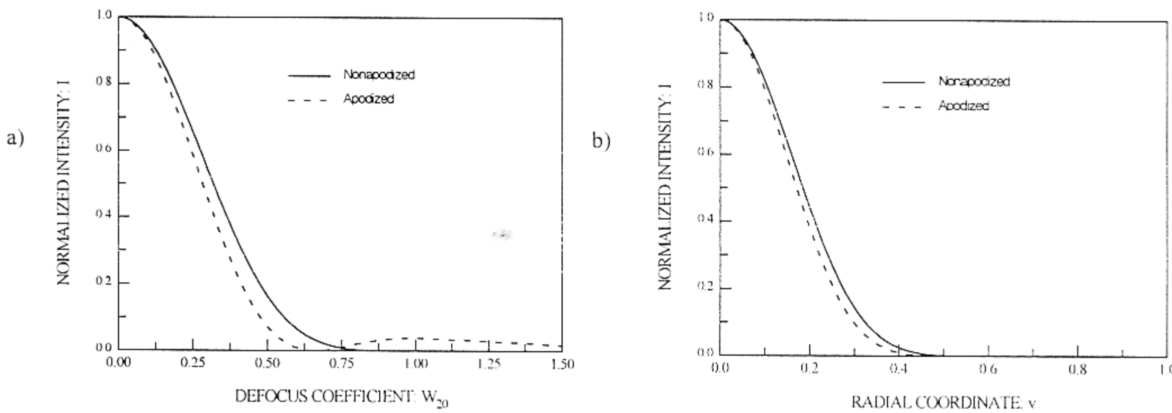

Fig.3. a) Normalized axial intensity PSF of: a nonapodized CSM (solid line), a CSM in which the illuminating and collecting sets are apodized by the filters $\tilde{p}_1(\rho) = \rho^2$ and $\tilde{p}_2(\rho) = 4\rho^4 - 4\rho^2 + 1$, respectively (dashed line); b) Normalized transverse intensity PSF for the same cases.

6. ACKNOWLEDGMENTS

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