Asymmetric apodization in confocal scanning systems

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A new class of superresolution pairs of pupil filters for three-dimensional, two-pupil confocal imaging is proposed. A distinctive feature of these filters is the asymmetry of their impulse response. For synthesizing the amplitude transmittance of such filters the Fourier transform properties of Hermitian functions are employed. It is shown that, with simple phase-only filters that belong to the class in question, either axial or unidirectional lateral superresolution is achieved. © 1998 Optical Society of America


1. Introduction

In the vast majority of cases the point-spread function (PSF) of an aberration-free optical imaging system is rotationally symmetric or it has at least two axes of symmetry. Nevertheless, in some systems the PSF can be made asymmetric on purpose. For example, Cheng and Siu¹ and Siu et al.² used this technique to achieve suppression of the sidelobes in the PSF (apodization). Here we use asymmetric PSF’s in subsystems of a confocal imaging arrangement to narrow the central maximum in the PSF of the whole system (superresolution). In what follows, we use the term apodization in a wide sense, i.e., for any modification of the pupil function (coherent transfer function) aimed at molding the PSF, unless it would lead to ambiguity.

From Fourier transform theory we know that to obtain an asymmetric PSF it is convenient to use either Hermitian or anti-Hermitian pupil functions.³ In fact, Cheng and Siu¹ and Siu et al.² chose highly Hermitian pupil functions to apodize both slit and circular pupils, although they did not describe them as Hermitian. In Refs. 1 and 2 it was shown that asymmetric PSF’s have a good side in which low sidelobes and a steep principal maximum are achieved at the cost of their worsening on the bad side. Similarly, as we see below, asymmetric superresolution PSF’s have their good and bad sides.

In this paper we show that, for some Hermitian pupils placed in both the illuminating and the collecting sets of a confocal imaging system, the problem of a bad side can be considerably relieved. It is also shown that, when we deal with the axial behavior of the three-dimensional (3-D) PSF of a confocal system, this function can be made symmetric and both of its sides are good, irrespective of the fact that PSF’s of the collecting and the illuminating sets are asymmetric functions of the axial coordinate. To demonstrate this, we propose certain Hermitian superresolution pupil functions.

2. Lateral Apodization

In this section we consider a coherent (i.e., with a point detector) confocal imaging arrangement that is a typical scheme of, for example, a confocal scanning microscope.⁴ In Fig. 1 a basic version of such an arrangement, viz., a transmission-mode microscope, is shown. A point source S of a narrow-band light of wavelength λ, e.g., a TEM₀₀ laser beam focused on a pinhole, is imaged by an illuminating system, which consists of lenses L₁₁ and Lₑₑ and a pupil filter P₁, onto an object O. The object is characterized by its amplitude transmittance t(x₀, y₀). The collecting system Lₑₑ, L₂₂, and P₂ images the illuminated spot of the object in the conjugated detector plane (x₂, y₂). When the object is scanned in the (x₀, y₀) plane the signal detected by a point detector D placed on the axis is processed by corresponding electronics and software, and finally the image of the object is displayed on a TV monitor. The spatial resolution of the image depends on both the PSF h₁₁(x₀, y₀) of the illuminating system and the PSF h₂₂(x₂, y₂) of the
collecting system. The PSF’s \( h_1 \) and \( h_2 \) are as follows:

\[
h_1(x_0, y_0) = \int \int P_1(\xi_1, \eta_1) \times \exp \left[ \frac{i}{f} (\xi_1 x_0 + \eta_1 y_0) \right] d\xi_1 d\eta_1, \quad (1)
\]

\[
h_2(x_2, y_2) = \int \int P_2(\xi_2, \eta_2) \times \exp \left[ -\frac{i}{f} (\xi_2 x_2 + \eta_2 y_2) \right] d\xi_2 d\eta_2, \quad (2)
\]

where \( P_1(\xi_1, \eta_1) \) and \( P_2(\xi_2, \eta_2) \) are the pupil functions, i.e., the amplitude transmittances, of pupil filters \( P_1 \) and \( P_2 \), respectively, \( k = 2\pi/\lambda \) is the wave number of the radiation, and \( f \) is the focal length. Let us assume that during the scanning process the point \((x_0 = x_s, y_0 = y_s)\) of the original, i.e., untransluted, object is placed on axis. Thus the origin of the object is now at the point \((x_0 = -x_s, y_0 = -y_s)\), and the object is described by the function \( t(x_0 + x_s, y_0 + y_s) \). In this position the amplitude distribution \( U(x_2, y_2) \) in the detector plane of the transmission-mode system can be written as the convolution of the amplitude in the object plane \( h_1(x_0, y_0)t(x_0 + x_s, y_0 + y_s) \) with \( h_2(x_2, y_2) \):

\[
U(x_2, y_2; x_s, y_s) = \int \int h_1(x_0, y_0)t(x_0 + x_s, y_0 + y_s) + h_2(x_2 - x_0, y_2 - y_0)dx_0 dy_0, \quad (3)
\]

where the unit magnification of the system is assumed. After a change of variables, \(-x_0, -y_0 \rightarrow x_0, y_0\), the intensity detected by a point detector at \( x_2 = y_2 = 0 \) is

\[
I(x_s, y_s) = \int \int |h_1(-x_0, -y_0)t(-x_0 + x_s, -y_0 + y_s) \times h_2(x_0, y_0)dx_0 dy_0|^2. \quad (4)
\]

With the definition

\[
h_1(x_0, y_0) = h_1(-x_0, -y_0)
\]

\[
= \int \int P_1(\xi_1, \eta_1) \exp \left[ -\frac{i}{f} (\xi_1 x_0 + \eta_1 y_0) \right] d\xi_1 d\eta_1, \quad (5)
\]

i.e., with both \( h_2 \) and \( h_1 \) defined as minus Fourier transforms of the corresponding pupil functions, the detected intensity becomes

\[
I(x_s, y_s) = \int \int h_1(x_0, y_0)t(x_0 - x_s, y_0 - y_s) \times h_2(x_2, y_2)dx_0 dy_0^2
\]

\[
= |h_1(x_s, y_s)h_2(x_s, y_s)|^2, \quad (6)
\]

where the symbol \( \otimes \) denotes the convolution operation. Relation (6) was derived previously under the assumption that \( h_q(q = 1, 2) \) are even functions (e.g., Ref. 5). In view of the above reasoning this
assumption seems superfluous. From Eq. (6) we can see that the image intensity of a point object, e.g., a pinhole in an opaque screen, described by a Dirac delta function, i.e., the PSF of the whole confocal system, is given by

$$\text{PSF}(x_s, y_s) = |h_1(x_s, y_s)h_2(x_s, y_s)|^2.$$  (7)

The same result holds for the reflection-mode system. Because of factorization of the amplitude impulse response (AIR) into $h_1$ and $h_2$, one has more freedom to shape the PSF of the confocal scanning system than with a classical system. Also, in the case of confocal fluorescence microscopy, in which the illuminating beam induces the fluorescence of an autofluorescent or suitably prepared object, a similar factorization takes place. As a result of the incoherence of the fluorescent light, we have

$$I(x_s, y_s) = |h_1(x_s, y_s)h_2(x_s, y_s)|^2 \otimes F(x_s, y_s),$$  (8)

where $F(x_s, y_s)$ stands for the spatial distribution of the fluorescence generation and, for simplicity, we assume that the wavelength of the fluorescent light equals that of the illuminating beam.

If $P_1$ and $P_2$ form a pair of complex-conjugated Hermitian pupil functions, i.e., $P_1^* = P_2$, Re$P_q$ is even, and Im$P_q$ is odd, then $h_q$ are real and asymmetric, and the following holds:

$$h_1(x_s, y_s)h_2(x_s, y_s) = h_1(-x_s, -y_s)h_1(x_s, y_s),$$  (9)

which means that for such a pair of pupil filters the PSF of the confocal system is symmetric with respect to the $x$ and $y$ axes.

To proceed further, we specify the form of $P_q$, as follows:

$$P_q(x_s, y_s) = \{H(x_s)\exp[-(1)^i\phi] + H(-x_s)\} \times \exp[-(1)^i\phi] (\text{circ} \frac{r_y}{r_0} - \text{circ} \frac{r_x}{\epsilon r_0}) + \text{circ} \frac{r_y}{\epsilon r_0},$$  (10)

where $H$ is a Heaviside unit step function, $\phi$ is the phase shift involved ($-\pi < \phi < \pi$), $r_0$ is the radius of the pupil, $r_q = (x_q^2 + y_q^2)^{1/2}$, and $\epsilon$ is a dimensionless coefficient ($0 < \epsilon < 1$). Thus we consider phase-only three-level pupils with a real inner disk and complex-conjugated outer semiannuli (Fig. 2). The same geometry for the pupil was assumed by Cheng and Siu. For such pupils the functions $h_q$ can be calculated analytically by means of known formulas for the Fraunhofer diffraction by a semicircular aperture. Nevertheless, we found that numerical calculation by means of the fast Fourier transform was more practical, as the closed-form result is expressed in terms of incomplete Struve functions that are not included in standard mathematical packages such as, for example, MATLAB or MATHEMATICA.

With the trial-and-error method, we found the phase shift of $\phi = \pi/3$ to be one for which the super-resolution of the system can clearly be demonstrated. To choose $\epsilon$, we drew $h_1^2$ and $(h_1h_2)^2$ as the functions of two variables: $x_s$ and $\epsilon$ for $y_s = 0$ and $\phi = \pi/3$. For each value of $\epsilon$, the cross sections of the PSF’s were normalized so that they reached unity at the absolute-maximum point. The gray-scale representations of those functions are shown in Fig. 3. It can be seen that the asymmetry of $h_q$ increases as $\epsilon$ decreases and that a value of $\epsilon$ equal to approximately 0.7 results in a noticeable narrowing of the central maximum of $(h_1h_2)^2$ and in small sidelobes. In Fig. 4 the main cross sections of $h_1^2(x_s, y_s; \pi/3, 0.7)$ and $h_1^2(x_s, y_s; \pi/3, 0.7)h_2^2(x_s, y_s; \pi/3, 0.7)$ are presented. It can be seen that, unlike in nonconfocal systems, the use of Hermitian pupils in confocal ones yields a PSF that has two perpendicular axes of symmetry. Along one axis the system demonstrates the desired properties, whereas along the other the system behaves in an even worse way than the nonapodized one. Thus here we should not speak of the good and the bad side of the PSF but rather of the good and the bad directions. In other words, we have two good sides and two bad sides. It should be noted that the above is not an inherent feature of any confocal system with Hermitian pupils. Such behavior of the system should be attributed to the fact that we chose a pair of complex-conjugated pupils. For example, for Hermitian pupils such that $P_1 = P_2$, the PSF of the confocal system will be asymmetric.

In the particular case of the pupil functions defined by Eq. (10) in which $\phi = \pi/3$ and $\epsilon = 0.7$, superresolution along the $x$ direction and small losses of energy (the Strehl ratio equals approximately 0.31) are experienced. Therefore the system could be especially useful for the imaging of highly directional patterns, e.g., diffraction gratings or muscle tissue. The superresolution properties of the confocal system in which the proposed pupil filters are placed are pre-
presented in Fig. 5, which shows images of two point sources of the fluorescent radiation separated by a normalized distance \( d \) and compares them with those for a nonapodized system. The resultant intensity is summed up on an intensity basis. It can be seen that, for two incoherent point sources located along the good direction and separated by the classical Rayleigh distance \( d = 0.610 \), the value of the intensity in the central minimum is considerably smaller than approximately 0.8, which is typical for nonconfocal systems. This occurs for both apodized and nonapodized systems. Such an advantage in spatial resolution is typical for confocal scanning imagery. For a confocal system that is apodized with the pupil filters proposed here, the value of 0.8 is achieved for values of \( d \) as small as 0.375.

3. Axial Apodization

A distinctive feature of coherent confocal imaging is its ability to provide 3-D images of 3-D objects. Therefore the axial behavior of a 3-D PSF is of interest because it represents a certain measure of the spatial resolution along the axis of the system. Also, this resolution can be improved by means of adequately designed pupil filters. As the axial behavior of the 3-D PSF is governed by the zero-order circular harmonic of the pupil function, which for a rotationally symmetric pupil is identical to the pupil function itself, we can restrict the analysis to rotationally symmetric pupils without loss of generality.

Let us introduce a normalized radial coordinate \( r \) in the pupil planes and cylindrical optical coordinates \((u, v)\) in the 3-D confocal region as follows:

\[
\begin{align*}
\rho &= r \sin \alpha, \\
v &= k(z_1 - z_2) \sin^2(\alpha/2), \\
u &= 4kz_1 \sin^2(\alpha/2),
\end{align*}
\]

where \( \alpha \) is the angular aperture of the illuminating and the collecting optics and \( z_1 \) is the actual axial scanning coordinate whose origin coincides with the confocal point. The 3-D AIR \( h_1(v, u) \) of the illuminating set is related to the pupil function by the following integral transform:

\[
h_1(v, u) = 2 \int_0^1 P_1(\rho_1) \exp \left( \frac{1}{2} iu \rho_1^2 \right) J_0(v \rho_1) \rho_1 \, d\rho_1.
\]

The relation that defines the 3-D AIR of the collecting set depends on whether we deal with a transmission-mode or a reflection-mode system:

\[
h_2(v, u) = 2 \int_0^1 P_2(\rho_2) \exp \left( + \frac{1}{2} iu \rho_2^2 \right) J_0(v \rho_2) \rho_2 \, d\rho_2.
\]
for a reflection system and

\[ h_d(v, u) = 2 \int_0^1 P_2(p_2) \exp \left( -\frac{1}{2} i u p_2^2 \right) J_0(v p_2) p_2 dp_2 \]  

(16)

for a transmission system. The 3-D PSF, i.e., the 3-D image of a point object, is given by

\[ \text{PSF}(v, u) = |h_1(v, u)h_2(v, u)|^2. \]  

(17)

For a reflection-mode system with two complex-conjugated pupils, Eqs. (14), (15), and (17) yield

\[ \text{PSF}_r(v, u) = |h_1(v, u)h_1^*(v, -u)|^2 \]

\[ = |h_1(v, u)h_1(v, -u)|^2. \]  

(18)

To maintain the relation of Eq. (18) in a transmission-mode system, i.e.,

\[ \text{PSF}_t(v, u) = |h_1(v, u)h_1(v, -u)|^2, \]  

(19)

we have to use a pair of identical pupil filters, which is produced by Eqs. (14), (16), and (17). To analyze the axial behavior of the PSF, and the PSF, we use a value of \( v = 0 \) in relations (18) and (19). In particular, we have

\[ \text{PSF}_r(u) = \text{PSF}_t(u) = |h_1(u)h_1(-u)|^2, \]  

(20)

where \( h_q(u) = h_q(0, u) \). To benefit from the above factorization and from properties of the Hermitian functions, we need to establish a Fourier transform relation between the pupil functions and the corresponding axial AIR's. This can be done with the following mapping in the pupil planes:

\[ \rho_q^2 - \frac{1}{2} = s_q. \]  

(21)

With the mapping of Eq. (21) and with \( v = 0 \), we can rewrite Eq. (14) as

\[ h_1(u) = \int_{-0.5}^{0.5} g_1(s_1) \exp(iu s_1/2) ds_1, \]  

(22)

in which an unessential phase factor is neglected and \( g_1(s_1) = P_1[h_1(s_1)] \) is a mapped pupil function that is
identically equal to zero for $|s_1| > 0.5$. Equations (20) and (22) yield
\[
\text{PSF}_i(u) = \text{PSF}_i(u) \\
= \left[ \int_{-0.5}^{0.5} g_1(s_1) \exp(\pm ius_1/2) ds_1 \right] \times \left[ \int_{-0.5}^{0.5} g_1(s_2) \exp(-ius_2/2) ds_2 \right]^2.
\]

Therefore we express the axial behavior of the 3-D PSF in terms of the Fourier transform of $g_1(s)$. Thus in the analysis to follow we consider such pupil functions $P_1(\rho)$ that become Hermitian when the mapping of Eq. (21) is completed. It is worth noting that the Fourier transform relation of Eq. (22) allows us to apply the results obtained previously for one-dimensional (1-D) asymmetric lateral apodization (slit pupils)\textsuperscript{1,2} to axial apodization, as there is a direct correspondence between mapped pupil functions $g(s)$ and 1-D actual pupil functions.

To design a superresolution pupil function $P_1(\rho)$ properly, we should take into account the following relations between $g(s)$ and $h(u)$, which hold for any Hermitian $g(s)$:
\[
h(0) = \int_{-\infty}^{\infty} g_o(s) ds,
\]
\[
\frac{\partial h(u)}{\partial u} \bigg|_{u=0} = -\frac{1}{2} \int_{-\infty}^{\infty} g_o(s) ds,
\]
where $g_r(s)$ and $g_o(s)$ are the real and the imaginary parts of $g(s)$, respectively, with $g_r(s)$ an even and $g_o(s)$ an odd function. As $P_1(\rho)$ must be a legitimate pupil function, we have to add the restriction
\[
g_r^2(s) + g_o^2(s) \leq 1.
\]

Good superresolution performance of $h(u)h(-u)$ can be attributed to a high value of the first derivative of $h(u)$ at the origin, hence by virtue of Eq. (25) to the first moment of $g_o(s)$, which favors a concentration of
$g(s)$ close to $s = \pm 0.5$, whereas the brightness in the image of a point object is directly proportional to the average value of $g(s)$. Maximum brightness is obtained for $g(s) = 0$ for which $h(u)$ takes a zero slope at the origin. Similarly, the maximum slope is obtained for $g(s) = s\text{sign}(s)\text{rect}(s)$, for which $g(s) = 0$ and $h(0) = 0$. Thus the requirement of obtaining high brightness and good superresolution is contradictory, and certain compromises should be established.

Taking into account the above considerations and the requirement of technological simplicity, we propose the following mapped phase-only three-level pupil function $g_1(s)$:

$$g_1(s) = \begin{cases} i & -0.5 \leq s < -0.25 \\ 1 & -0.25 \leq s < 0.25 \\ -i & 0.25 \leq s \leq 0.5 \\ 0 & \text{otherwise} \end{cases}$$  \hspace{1cm} (27)$$

The actual three-zone pupils $P_1$ and $P_2$ generated by $g_1(s)$, which should be used in a reflection-mode system, are shown in Fig. 6. The axial behavior of the impulse response of a conventional imaging system with a pupil function $P_1$ and that of a two-pupil confocal scanning system are presented in Figs. 7(a) and 7(b), respectively. It is seen that improvement of the axial resolution is accompanied by an increment of the sidelobes, which is a usual effect observed in superresolution systems. Using the full width at half-maximum (FWHM) as a merit function, we can state that, by means of simple Hermitian pupils, one can achieve a 30% gain in axial resolution.

Another important characteristic of confocal imaging is its capacity for sectioning. In the case of a transmission-mode system the so-called integrated intensity is a measure of this capacity. The integrated intensity is defined as follows:

$$I_{\text{int}}(u) = \int_{-\infty}^{\infty} |h_1(u, v)h_2(u, v)|^2\text{d}v.$$  \hspace{1cm} (28)$$

Making use of Eqs. (14), (16), (21), and (27), we calculated numerically the integrated intensity for a transmission-mode system with two pupils $P_1'$. The
result is presented in Fig. 7(c). A 30% gain in the sectioning capacity (in terms of the FWHM) can be seen. In reflection-mode systems, we use instead the confocal signal \( I(u) \) and not the integrated intensity. The confocal signal is, by definition, the signal received by the point detector when a perfect reflector perpendicular to the axis of the illuminating system moves axially in the vicinity of the confocal point. This magnitude can be expressed in terms of mapped pupil functions as follows:

\[
I(u) = \int_{-0.5}^{0.5} g_1(s)g_2(s)\exp(\text{i}us)\,ds \quad . \tag{29}
\]

Because \( g_1(s) = g_2^*(s) \) and \( |g_1(s)| = |g_2(s)| = 1 \), Eq. (29) reduces to that for the nonapodized system. Thus no gain in confocal signal is obtained. This holds for any pair of complex-conjugated purely phase pupils. On the other hand, it can be shown that, for confocal fluorescence imaging, the confocal signal is identical to the integrated intensity.15 This is because in the latter case we use the concept of a uniform-fluorescence flat surface perpendicular to the axis and not that of a perfect reflector. Therefore in 3-D confocal fluorescent imaging we benefit from the improved sectioning capacity of the proposed pupil filters in both transmission- and reflection-mode systems.

4. Discussion and Conclusions

The superresolution achieved with the two specific filters proposed here is significant, although their pupil functions were not optimized in a rigorous manner. Therefore further improvement concerning the reduction of the spot size of the PSF or an increase of the Strehl ratio is expected. It is worth noting that, for the pupil filters in question, an optimization procedure should take into account the following constraints, which do not appear in classical apodization problems: an optimized AIR of the confocal system must be a product of two real, asymmetric, but mutually symmetric, functions.

The asymmetric lateral PSF's considered here and in Refs. 1 and 2 resemble, to some extent, the PSF of a system with the aberration of coma. On the other hand, there are some essential differences between coma and asymmetric apodization. For example, coma does not affect the images of axial points, whereas asymmetric apodization does. Similarly, the asymmetry of the axial PSF should not be considered an aberration of defocus, unless one considers mapped pupil functions of the form \( g(s) = \cos(\text{AS}_a) + i\sin(\text{AS}_a) \), which are Hermitian and by virtue of Eq. (21) yield the actual pupil functions \( P(\rho_0) = \exp(-\text{i}A/2)\exp(\text{i}A\rho_0^2) \). In a defocused system the axial behavior of the PSF remains symmetric about the actual focus, whereas in general this is not the case if asymmetric axial apodization is concerned [Fig. 7(a)]. Nevertheless, the above remarks confirm the existence of a close relation between aberrations and apodization,16 especially if one deals with phase-only apodizers. Moreover, even in nonconfocal systems some aberrations can cause the same effects as do superresolution filters.17

In a recent study by Sales and Morris18 it was shown that multilevel phase-only superresolution diffractive elements not only benefit from advances made in diffractive optics technology but also demonstrate many advantages with respect to other super-resolution elements. It is worth mentioning the flexibility of design, the simplicity of the optimization procedures, superresolution performance, and tolerances to fabrication errors. Thus our choice of using phase-only three-zone three-level pupils, which we made with partially heuristic reasoning, finds additional justification.

In view of our results and those presented in Refs. 1 and 2, it seems that the most promising area of application of asymmetric apodization is two-pupil confocal scanning systems and, in particular, confocal microscopes.

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