The Point Spread Function (PSF) using Longitudinal Black and White Strips inside a Circular Aperture

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(Received 04th January, 2017; Revised 26th February, 2017; Accepted 13th March, 2017; Published: 26th March, 2017)

Abstract- We have selected two different apertures one in the form of concentric unequal ratio of annuli and the second has longitudinal successive black and white strips made inside a circular aperture. We compute the Point Spread Function (PSF) in the two cases for the sake of resolution improvement. In addition, the Resultant PSF is obtained in case of confocal imaging. A comparison of the PSF with circular and annular apertures is made. In addition we have computed the coherent transfer function (CTF) for the confocal scanning laser microscope (CSLM) using the above models. The effect of depth of focus for the considered B/W concentric annuli is investigated. Erythrocytes blood images using the above models are processed based on Fourier transform techniques and using Mat-Lab code.

Key Words: longitudinal black and white strips, Coherent Scanning Laser Microscope, image processing of Erythrocytes blood images.

1. INTRODUCTION

Several theoretical publications upon the confocal microscope are reported in many articles since 1977 [1-13] to investigate the microscope. In addition, separate work upon many modulated apertures is made since 1983 [14-18] and others for the sake of lateral and axial resolution improvement. Recent publication investigates the combined linear-quadratic aperture [19] gain improvement in the PSF and hence in lateral resolution as compared to the circular apertures. An application of Hamming aperture upon confocal microscope is investigated in [20]. Three-dimensional imaging of neurons by application of Hamming aperture upon confocal microscope are reported in many articles since 1977 [1-13] to investigate the microscope. In addition, separate work upon many modulated apertures is made since 1983 [14-18] and others for the sake of lateral and axial resolution improvement.

In this manuscript, we investigate two apertures one of concentric unequal annuli that have a ratio of 2:1 from the center, while in the other aperture of longitudinal B/W equal strips truncated by the circular aperture. An application of Hamming aperture upon confocal microscope is investigated in [20]. Three-dimensional imaging of neurons by application of Hamming aperture upon confocal microscope are reported in [23-28].

In this study, we have selected two different apertures one in the form of concentric unequal ratio of annuli and the second has longitudinal successive black and white strips made inside a circular aperture. We compute the Point Spread Function (PSF) in the two cases for the sake of resolution improvement. In addition, the Resultant PSF is obtained in case of confocal imaging. A comparison of the PSF with circular and annular apertures is made. In addition we have computed the coherent transfer function (CTF) for the confocal scanning laser microscope (CSLM) using the above models. The effect of depth of focus for the considered B/W concentric annuli is investigated. Erythrocytes blood images using the above models are processed based on Fourier transform techniques and using Mat-Lab code.

2. ANALYSIS

Two different models of apertures are considered for modulated apertures in order to gain compromised resolution and contrast improvements. We suggest the familiar concentric annuli suggested early by A.M. Hamed, and J.J. Clair in [15] but of unequal cascaded black and white (B/W) annuli in a ratio of 2:1 from the center, or in the form of longitudinal B/W equal strips truncated by the circular aperture.

2.1. First model of B/W unequal annuli of a ratio 2:1 from the center:

This aperture is composed of B/W unequal concentric annuli starting from the center as black. The ratio between the black and transparent annuli is 2:1. The running letter I varies from 1 up to 4 in the presented model.

\[ P_{1st\ model}(u, v) = \sum_{i=1}^{N} \left[ P_{3i}(3i \frac{\rho}{\rho_0}) - P_{3i-1}(3i - 1 \frac{\rho}{\rho_0}) - 1 \frac{\rho}{\rho_0}) \right] \quad (1) \]

Where \( \rho = \sqrt{u^2 + v^2} \) is the radial coordinate in the aperture plane of orthogonal coordinates \( (u, v) \), \( \rho_0 \) is the total radius, and \( N \) represent the total number of transparent segments. The relation of the coordinates \( u \) and \( v \) and the radial coordinate \( \rho \) is governed by this transformation:

\[ u = \rho \cos(\theta) \text{ and } v = \rho \sin(\theta). \]

The azimuthal angle \( \theta \) represents the azimuthal coordinate.

Hence, for the difference between two successive circles gives an annular shape computed from the following difference:

\[ P_{3i}(3i \frac{\rho}{\rho_0}) - P_{3i-1}(3i - 1 \frac{\rho}{\rho_0}) \]

e.g. for \( i = 1 \), we write the internal transparent annulus as follows:

\[ P_{3}(3 \frac{\rho}{\rho_0}) - P_{2}(2 \frac{\rho}{\rho_0}) \]

While the dark central circle zone occupies \( P_{2}(2 \frac{\rho}{\rho_0}) \).
In this model, \( N = 4 \) stands for the four transparent segments, equation (1) rewritten as follows:

\[
P_{1\text{st model}}(u, v) = P_3 \left( \frac{\rho}{\rho_0} \right) - P_2 \left( 2 \frac{\rho}{\rho_0} \right) + P_6 \left( 6 \frac{\rho}{\rho_0} \right) - P_5 \left( 5 \frac{\rho}{\rho_0} \right) + P_9 \left( 9 \frac{\rho}{\rho_0} \right) - P_8 \left( 8 \frac{\rho}{\rho_0} \right) + P_{12} \left( 12 \frac{\rho}{\rho_0} \right) - P_{11} \left( 11 \frac{\rho}{\rho_0} \right)
\]  

This model has successive black and transparent concentric annuli where the number of transparent annuli \( N = 4 \) is shown as in the Fig. (1- a).

Since the aperture has circular symmetry of revolution, then it is independent on the azimuthal coordinate, namely the angle \( \theta \). \( r = (x, y) \) is the radial coordinate in the Fourier plane located in the focal plane of the Fourier transform lens. \( f \) being the focal length of the Fourier transform lens at wavelength of illumination \( \lambda \).

Substitute from equation (2) in equation (3), and operate the transformation we finally get the PSF as follows:

\[
h_{1\text{st model}}(r) = \text{const.}\left\{ \left[ J_1(0.25 z) - J_1(0.17 z) \right] - \left[ J_1(0.5 z) - J_1(0.42 z) \right] + \left[ J_1(0.75 z) - J_1(0.67 z) \right] + \left[ J_1(0.92 z) \right] \right\}
\]  

In equation (4), \( J_1 \) is the Bessel function of the 1st order and \( z \) is the reduced coordinate related to the radial coordinate in the Fourier plane by the following relation: \( z = r \left( \frac{2 \pi}{\lambda f} \right) \rho_0 \) and \( \rho_0 \) is the radius of the aperture.

It is known that NA is the numerical aperture of the objective lens where \( \rho_0 \) is the total radius of the aperture.

2. Second model of longitudinal B/W strips truncated by a circular aperture:

This aperture is fabricated by the multiplication of two dimensional rectangular matrix composed of longitudinal B/W strips with a circular aperture of radius \( r \).

\[
P_{2\text{nd model}}(u, v) = P_{\text{cir}}(u, v). P_{\text{comb}}(u, v) \tag{5}
\]

Where

\[
P_{\text{cir}}(\rho) = \begin{cases} 1, & |\frac{\rho}{\rho_0}| \leq 1 \\ 0, & \text{elsewhere} \end{cases} \tag{6}
\]

\( \rho = (u, v) \) is the radial coordinate in the aperture plane and \( \rho_0 \) is the aperture radius.

And,

\[
P_{\text{comb}}(u, v) = \sum_{n=0}^{N} \text{rect}\left( \frac{(u - (2n + 1) u_d)}{u_0} , v \right) \tag{7}
\]

Where \( u_0 \) is the rectangular width while \( u_d \) is the distance between two successive B/W rectangles. \( N \) is the total number of strips.

Consequently, the fabricated aperture is written, considering \( u_0 \) the rectangle width is normalized as in equation (8), as follows:

\[
P_{2\text{nd model}}(u, v) = P_{\text{cir}}(\rho) \cdot \sum_{n=0}^{N} \text{rect}\left( \frac{(u - (2n + 1) u_d)}{u_0} , v \right) \tag{8}
\]

Where \( \text{rect}(u) = 1, \text{for} |\frac{u}{u_0}| \leq 1 \)

The Point Spread Function (PSF) is computed by operating the Fourier transform upon equation (8), we write:

\[
h(r) = \text{F.T.} \{ P_{2\text{nd model}}(u, v) \} \tag{9}
\]

Hence,

\[
h(r) = \text{F.T.} \{ P_{\text{cir}}(\rho) \cdot \sum_{n=0}^{N} \text{rect}\left( \frac{(u - (2n + 1) u_d)}{u_0} , v \right) \} \tag{10}
\]

Making use of convolution operation [21, 22], the PSF given by equation (10) becomes:
Hence, the aperture given by equation (5) is rewritten as functions by a comb of spikes of Dirac-Delta functions.

For a real modulated aperture, it is limited by the diameter of the circle surrounding the strips.

For simplicity, consider a real two dimensional annuli to replace the above one dimensional strip arrangement then we write:

\[
h(r) = \frac{2J_1(\alpha r)}{\alpha r} \otimes \sum_{k=-\infty}^{\infty} \delta(x - \frac{k}{a})
\]  

(17)

After making use of convolution operation, we get finally this equation for the PSF:

\[
h(r) = \sum_{k=-\infty}^{\infty} 2J_1(\alpha (x - \frac{k}{a})^2 + y^2)^{1/2}
\]

(18)

Another solution to equation (19) is obtained considering mono-dimensional problem since the strips of finite width are constant along y = 0, then equation (19) is reduced as follows:

\[
h(x; y = 0) = \sum_{k=1}^{4} 2J_1(\alpha (x - \frac{k}{a})^2 + y^2)^{1/2} = \sum_{k=-\infty}^{\infty} 2J_1[\alpha (x - \frac{k}{a})]
\]

(21)

The RPSF (h_\text{r}) in case of CSLM is the given by the simple product of both of the PSF corresponding to the two objectives of the microscope.

\[
h_\text{r}(r) = h_{\text{1st model}}(r). h_{\text{2nd model}}(r) ; \text{for the 1st model}
\]

A similar expression is given for the RPSF in case of the 2nd model.

Consequently, the image of a point in case of the CSLM is the 4\text{th} power of h_\text{r} as compared with the modulus square value of h_\text{1} or h_\text{2} for symmetric modulated apertures.

2.3. Computation of the Coherent Transfer Function (CTF) using CSLM

It is known that the CTF is computed for the CSLM from the convolution product of the objective lenses of the confocal arrangement since both objectives contribute equally to the resolution of the microscope. Hence, the CTF is computed as follows:
The computation of equation (16) corresponding to the above two models described in equations (2, 5) is realized directly or by using the Fourier transform (F.T.) techniques [21, 22]. Consequently, the Resultant PSF is obtained by operating the F.T. upon equation (16) to get the results outlined in equations 4 and 14 as follows:

\[
h_{\hat{1}st\ model}(x,y) = F.T.\left[ C(u,v) \right] = F.T.\{ P_1(u,v) * P_2(u,v) \} = h_{\hat{1}st\ model}(x,y)\]

(24)

Then we operate the inverse FT upon equation (17) to get the CTF. These two cascaded F.T. operations are operated using the MatLab code in order to compute the CTF.

2.4. The effect of depth of focus

The defect of focus for ordinary optical system is investigated by different authors, and a new formula investigated by [29] is extended in our model of B/W concentric annuli giving this formula

\[
\Delta z_t = \lambda \sum_{i=1}^{N} \frac{1}{1 - \sqrt{1 - (\Delta NA_{2i})^2}}
\]

(25)

Where \(\Delta NA_{2i} = NA_{2i} - NA_{2i-1}\) represent the transparent annular width as shown in the Fig. (2) and \(\Delta z_t = \Delta z_1 + \Delta z_2 + \ldots\)

We considered independent concentric transparent annulus since they are completely separated.

3. RESULTS AND DISCUSSION

All results are computed at moderate numerical aperture NA= 0.5 and at certain wavelength \(\lambda = 500\) nm. All these curves are computed using the MatLab code.

The image of the 1st model is plotted as in the Fig. (1- a). It has concentric black and transparent annuli, where the number of black annuli = the number of transparent annuli = 4. The ratio of B/W annuli is 2:1 from the center, and the aperture radius = 256 pixels. The aperture has dimensions of 2048 \(\times\) 2048 pixels. The image of the 2nd model has concentric black and transparent annuli, where the number of black annuli = the number of transparent annuli = 4 while the ratio of B/W annuli is 8:1 from the center as shown in the Fig. (1- b).

The defect of focus in case of B/W concentric annuli is considered. Only four strips are considered for simplicity of the graph. The wave front defect of focus corresponding to this model is drawn as shown in the Fig. (2). The total numerical aperture is \(NA = n \sin \alpha\). The successive NA for the concentric annuli is \(\sin \alpha_1, \sin \alpha_2, \sin \alpha_3, \text{ and } \sin \alpha_4\) as shown in the Fig.. R is the wave-front radius and \(w\) is the aberration wave front shift.

Fig. 4-a: The image of the 2nd model. It has successive black and transparent longitudinal strips, where the number of black strips = the number of transparent strips = 6 and the strip width = 5 pixels. The aperture has dimensions of 256 \(\times\) 256 pixels and the circle radius = 32 pixels.

Fig. 4-b: The image of the 2nd model where the radius = 16 pixels.

Fig. 4-c: The image of the 2nd model where the radius = 8 pixels.
Grating used in the formation of longitudinal strips inside the circular aperture is plotted as in the Fig. (3). Strip width = 5 pixels and the matrix has dimensions of 256 × 256 pixels.

The image of the 2\textsuperscript{nd} model obtained from the multiplication of the rectangular grating and the uniform circular aperture is plotted as in the Fig. (4). It results successive black and transparent longitudinal strips, where the number of transparent strips = 6 at the circle radius = 32 pixels, as shown in the Fig. (4- a). In Fig. (4- b), the aperture radius = 16 covering only 4 transparent strips while in the Fig. (4- c), the aperture radius = 8 covering only 2 transparent strips. The aperture has dimensions of 256 × 256 pixels and the strip width remains the same in all plots at 5 pixels.

It is shown that, an improvement in resolution is attained using the confocal microscope provided with the modulated 1\textsuperscript{st} model where \( r_c = 757.6 \text{ nm} \) as shown from the results.

Further improvement is attained using the 2\textsuperscript{nd} model where \( r_c(\text{annular}) = 697 \text{ nm} \).

The PSF cut off spatial frequency corresponding to the first model is given by \( r_c = 757.6 \text{ nm} \), the minimum, while that corresponding to the circular aperture is the greatest and the first model lie intermediately between the circular and the annular apertures. Consequently, moderate resolution is attained using the first described model while the annular resolution is the optimum but at the expense of low light intensity transmitted from the annulus giving poorer contrast.
Secondly, the image contrast is improved in case of the described model since it gains more light transmitted from the cascaded annuli as compared with the annular aperture.

The cut off spatial frequency for some apertures are computed from the corresponding PSF as given in the table (1). The values of the cut-off spatial frequencies are in nm.

\[ r_c = \frac{\Delta r}{r_{\lambda}} \]

<table>
<thead>
<tr>
<th>Aperture Type</th>
<th>( r_c ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circular</td>
<td>787.9</td>
</tr>
<tr>
<td>Model 1</td>
<td>757.6</td>
</tr>
<tr>
<td>Model 2</td>
<td>697</td>
</tr>
<tr>
<td>Annular</td>
<td>333.3</td>
</tr>
</tbody>
</table>

Table (1)

![Fig. 7: PSF of the circular aperture as a function of the radial coordinate (r) in the Fourier plane. The cut-off spatial frequency is \( r_c = 787.9 \) nm.](image1)

![Fig. 8: PSF of the annular aperture as a function of the radial coordinate (r) located in the Fourier plane. The cut-off spatial frequency is \( r_c = 333.3 \) nm.](image2)

![Fig. 9: The plot of the 1st model and its corresponding PSF using reduced coordinates.](image3)

![Fig. 10: PSF of the model (2) where \( \Delta r = 0.02 \) as a function of the radial coordinate (r) in the Fourier plane. The cut-off spatial frequency is \( r_c = 697 \) nm. \( \Delta r \) is the radial width of concentric annuli assumed constant. Hence, \( \Delta r/r = (.23/(.25-.23)) = 11.5:1 \).](image4)

![Fig. 11: PSF of the model (2) considered as mono-dimensional strips where a = 25 pixels, and N = 6 as a function of the horizontal coordinate (x) in the Fourier plane. The cut-off spatial frequency is \( r_c = 101 \) nm. N is the number of strips.](image5)

![Fig. 12: Defect of focus versus the numerical aperture NA. The solid curve stands for the annular aperture, the red discontinuous curve for the circular aperture, while the third dotted curve stands for B/W concentric annuli with two transparent and two dark zones.](image6)
The PSF for the concentric annular aperture for different annular width where ratio of B/W annuli is 8:1 is plotted as shown in the Fig. (10). While in the Fig. (11), the normalized PSF is plotted for the mono-dimensional strips bounded by the circular aperture for strip spacing a=25 pixels using equation (21). The number of strips are taken as N= 6. It gives cut-off x = 101 pixels. This cut-off result in one dimension is not compared with the other cited results in radial coordinate.

Fig. 13: The image of the CTF for the 1st model and its plot is shown in the left side while the corresponding CTF image and its plot for circular aperture shown in the right side. The CTF is computed from the direct convolution of the two apertures.

Fig. 14: The image of the CTF for the 2nd model and its plot is shown in the left side while the corresponding CTF image and its plot for circular aperture shown in the right side. The CTF is computed from the direct convolution of the two apertures. The strip width = 10, and matrix dimensions is 512 x 512 pixels for the CTF while it is only 256 x 256 pixels for the modulated apertures.

The results of the Defect of focus versus the numerical aperture NA are plotted as shown in the Fig. (12). The solid curve stands for the annular aperture, the red discontinuous curve for the circular aperture, while the third dotted curve stands for B/W concentric annuli with two transparent and two dark zones. It is shown that greater values of Δz correspond to the annular aperture approximated by one transparent annulus and the smaller value corresponds to the circular aperture manipulation. Intermediate values of Δz are shown for B/W concentric arrangement of two transparent annuli.

The image of the CTF for the 1st model and its plot is shown in the left side while the corresponding CTF image and its plot for circular aperture shown in the right side as plotted in the Fig. (13). It is shown a harmonic variation inside the CTF corresponding to the 1st model as compared with the regular decaying shape given for the circular aperture. Another plot of the CTF is shown as in the Fig. (14) for the 2nd model of rectangular strips.

Fig. 15: Coherent Transfer Function (CTF) using two annular symmetric apertures. The total width is 256 pixels which equals two times the aperture diameter.

Fig. 16: Computation of the CTF for the 1st model using two different techniques, on the left direct convolution product is shown while on the right FT technique is applied as shown in the Fig. (16). The image of the Fourier model 1 using two different techniques is made, on the left direct convolution product is shown while on the right FT technique is applied as shown in the Fig. (16). The image of the Fourier

Fig. 17: The image of the Fourier spectrum originated from the circular aperture provided with straight lines of black and transparent strips. The Computation of the CTF for the 1st model aperture using two different techniques is made, on the left direct convolution product is shown while on the right FT technique is applied.

The CTF using two annular symmetric apertures is plotted as in the Fig. (15) for comparison. A central peak is shown and two small peaks are located the edges at [128, 384] corresponding to the annular aperture. The total width is 256 pixels which equals two times the aperture diameter as expected.

Fig. 17: The image of the Fourier spectrum originated from the circular aperture provided with straight lines of black and transparent strips.

The Computation of the CTF for the 1st model aperture using two different techniques is made, on the left direct convolution product is shown while on the right FT technique is applied as shown in the Fig. (16). The image of the Fourier spectrum...
spectrum originated from the circular aperture provided with straight lines of black and transparent strips is shown as in the Fig. (17).

![Fig. 18: Reconstructed image obtained from the original image using digital CSLM provided with two symmetric concentric annular apertures corresponding to the 1st model.](image)

The reconstructed image obtained from the original image using digital CSLM is shown as in the Fig. 18. The 1st model of apertures is applied in the getting of the reconstructed image. The Fourier transform techniques and MatLab codes are the basic tools of computations.

4. CONCLUSION

It is concluded as shown from the results, that: a) the PSF corresponding to the 1st model has a cut-off spatial frequency  \( r_c = 757.6 \) nm. b) While the cut-off spatial frequency corresponding to the 2nd model is  \( r_c = 697 \) nm. It is a two dimensional concentric annuli of a very small width which is considered as generalization of the mono dimensional transparent strips. c) Comparative results for the cut-off spatial frequencies corresponding to the circular and annular apertures gave the extreme values of  \( r_c \) (circle) = 787 nm and  \( r_c \) (annulus) = 333 nm.

We conclude that the resolution which is computed from the cut-off spatial frequency is improved considering the investigated two models as compared with the resolution of circular aperture. The contrast is better as compared with the annular aperture. Hence, a compromising of resolution and contrast is attained considering the described models. The application of these models upon the CSLM is made using blood images giving reconstructed images of better contrast than that manipulated with the annular apertures. The aperture composed of B/W strips is useful for imaging of extended objects since the PSF showed considerable harmonic peaks around the central peak as shown in the Fig. (6). In addition, the resolution of the CSLM is further improved as compared with circular apertures as shown from the results since the resultant PSF is equal to the multiplication of the two objectives forming the confocal microscope.

REFERENCES


