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Three-dimensional coherent transfer function in reflection-mode confocal microscopy with two-zone phase filters

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ABSTRACT

The three-dimensional (3D) coherent transfer function (CTF) in a reflection-mode confocal scanning microscope using two-zone phase filters has been derived analytically. The numerical results for various phase filters are presented and the characteristics of different cases are compared. Finally, the image sharpness expressed by in-focus CTF and the effect of Gaussian beam are discussed. More information in the high spatial frequencies can be transferred in both cases, which is normally at price of the degrading the axial resolution and the image sharpness, as expected. However, the axial resolution remains unchanged for two equal phase filters with the phase difference of π. In addition, the dark-field imaging may be produced in a system with one phase filter. It is also found that the contrast-reversal phenomenon would occur in focal plane and the maximum value of axial response is not at the origin in some cases.

1. Introduction

Reflection-mode confocal microscopy is extensively used in the field of materials and life sciences [1–3], because of the improvements of image contrast, resolution and the ability of gaining the axial information of samples [4]. Many methods have been studied to further enhance the resolution, including the pupil filtering method, which introduces a pupil filter into the entrance or exit pupil of a confocal system to change the intensity distribution of the Airy spot [5]. The amplitude pupil filter is most common used to increase the axial and transverse resolutions [6,7]. Much attention has been paid to research on the phase pupil filters to produce a sharper focal spot [8–10].

Various spatial features of confocal microscopy have been investigated using the point spread function (PSF) [11–13]. However, the PSF is not powerful enough to reveal the properties of optical systems in the frequency domain. It is not known whether the axial cut-off frequency is improved or how the transverse information transfer capability is affected. The three-dimensional (3D) coherent transfer function (CTF), as a useful tool to completely describe 3D confocal non-fluorescent imaging of thick samples, can describe the transfer capability at different frequencies to get the change of the resolution [14–16]. In addition, frequency analysis is useful when considering the characteristics of super-resolution imaging technology, because the transfer capability at high frequency affects image details (corresponding to high-resolution area) [17,18]. It is also informative to examine the low frequency components when comparing different CTFs, because these components are related to the sketch of a sample [19]. The CTF of confocal microscopy systems with amplitude pupil filters have been analyzed previously [20–22], while the CTF of a reflection-mode confocal scanning

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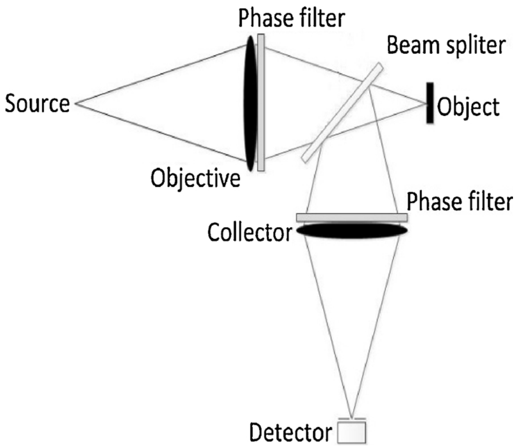

Fig. 1. The geometry of a reflection-mode confocal microscope with two-zone phase filters.

microscope using phase filters has never been studied to reveal its characteristics in the frequency domain.

In this paper, we investigate the reflection-mode confocal microscopy with two-zone phase filters based on diffraction optics and first derive the 3DCTF with a point source and a point detector. Then we analyze the axial resolution and transverse resolution through various 3D and two-dimensional (2D) numerical experiments. The comparisons between one filter, two equal filters and two unequal filters are also provided. Finally, we further investigate the image of a straightedge object to discuss the image sharpness in terms of 2D in-focus CTF and the effect of using Gaussian beam in the confocal microscopy.

2. Theory method

The geometry of a confocal microscope with a point source, a point detector and phase pupil filters can be given by Fig. 1. In such a system, we can express the image intensity of a thick object with amplitude reflectivity $t(x, y, z)$ as [16],

$$I(r_s) = \left| \int_{-\infty}^{\infty} c(m) T(m) \exp(2\pi i m \cdot r_s) dm \right|^2 \tag{1}$$

Where $r_s = (x_s, y_s, z_s)$ is the scan point, m represents the spatial frequency vector with two transverse components m and n, and there is one axial component s. $c(m)$ is the 3D amplitude CTF. $T(m)$ is the Fourier transform of $t(x, y, z)$.

Assuming the objective and collector lenses being aberration-free, and $c(m)$ can be expressed as,

$$c(l,s) = \int_{-\infty}^{\infty} c_2(l,u) \exp(-ius) du \tag{2}$$

Where $l = \sqrt{m^2 + n^2}$ denotes the radial spatial frequencies. $c_2(l, u)$ is the 2D defocused CTF given by,

$$c_2(l, u) = K \times P_1(l, u) \otimes_2 P_2(l, u) \tag{3}$$

Where $K = 2\pi/\lambda$ is a constant of normalization. P_1 and P_2 are the defocused pupil function of the objective lens and collecting lens, respectively. $\otimes_2$ represents the 2D convolution operation on the transverse plane. u denotes the normalized axial optical coordinate, related to the real defocus distance z, via,

$$u = 2\pi z/\lambda (d/f)^2 \tag{4}$$

Where d and f represent the radius and the focal length of the objective lens. λ is the wavelength of the illumination light.

As shown in Fig. 2, the two-zone phase filter with the inner radii $\varepsilon_j (0 < \varepsilon_j < 1)$ and the phase change $\phi_j (0 < \phi_j \le \pi)$ is utilized in such a reflection-mode confocal microscope. The defocused pupil function under the paraxial approximation can be expressed as,

$$P_j(\rho, u) = \begin{cases} \exp(iu\rho^2/2)\exp(i\phi_j) 0 \le \rho \le \varepsilon_j \\ \exp(iu\rho^2/2) \varepsilon_j < \rho \le 1 \end{cases} \tag{5}$$

Where j= 1,2 correspond to an objective lens with the filter and a collector lens with the filter, respectively. $\rho = r/d$ is the normalized radius of the lens pupil and $r = \sqrt{x^2 + y^2}$ denotes the real radial coordinate.

To be convenient, the pupil function with two-zone phase filter can be given by two circular pupil functions with the radii of 1 and ε_j, respectively, via,

$$P_j a(\rho, u) = \exp(iu\rho^2/2) 0 \le \rho \le 1 \tag{6}$$

$$P_{jb}(\rho, u) = \exp(iup^2/2) 0 \le \rho \le \varepsilon_j \tag{7}$$

Then the pupil function can be expressed as,