Improvement of Axial Resolution in Confocal Microscopy by an Optical Pupil Filter with Two Zones Phase-Shifted by π *

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A filter with two zones phase-shifted by π is proposed to improve the axial resolution of confocal microscopes with a finite-sized detector. The optimum axial resolution for a given size of the detector can be achieved by adjusting the zone boundary of the filter. The experimental results are well in agreement with the theoretical predictions.

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One of the most popular features of confocal scanning optical microscopy is its excellent depth discrimination and its three-dimensional (3D) object-reconstruction capability.[1] Axial resolution is significant for confocal microscopy where the high depth-discrimination feature allows the formation of 3D images by sectioning only a thin slice of a sample at a time. Several researchers have studied pupil filters in order to improve axial resolution.[2−9] However, most of them have only investigated the filters applied to confocal microscopes with a point detector. In practice, no detector is a single point but has a finite size, i.e., a finite-sized pinhole is usually placed in front of the detector. Also, the size of pinhole is sometimes increased further to raise the signal strength. In this Letter, we theoretically describe the property of a phase-only filter composed of two zones phase-shifted by π which is applied to confocal microscopes with a finite-sized detector. Theoretical analyses indicate that the improvement of the axial resolution can be obtained if the value of the detector is larger than a certain value. Furthermore, the optimum axial resolution for a given size of the detector can be achieved by altering the zone boundary of the filter. The experimental results for the improvement of the axial resolution are demonstrated, which is in good agreement with the theoretical analysis.

The most useful technique which serves to characterize the performance of a confocal microscope is to measure the detected signal as a plane reflector is scanned along the axial direction.[10] In terms of diffraction theory, this axial response can be derived as

$$I(u) = \int_0^{v_d} \left| \int_0^1 P(\rho) \exp(iu\rho^2) J_0(v\rho) \rho \mathrm{d}\rho \right|^2 v \mathrm{d}v, \quad (1)$$

where J_0 denotes the Bessel function of the first kind of the order of zero, $P(\rho)$ is the pupil function of the objective. The optical coordinates u and v are defined by

$$u = \frac{8\pi}{\lambda} z \sin^2 \frac{\alpha}{2} \quad (2)$$

$$v = \frac{2\pi}{\lambda} r \sin\alpha \quad (3)$$

Here λ is the illumination wavelength, and $\sin\alpha$ is the numerical aperture of the objective in object space. The upper limitation of the integration in Eq. (1)

$$v_d = \frac{2\pi}{\lambda} r_d \sin\alpha_d \quad (4)$$

denotes the normalized detector radius. Here r_d and $\sin\alpha_d$ are the real detector radius and the numerical aperture of the objective in image space, respectively.

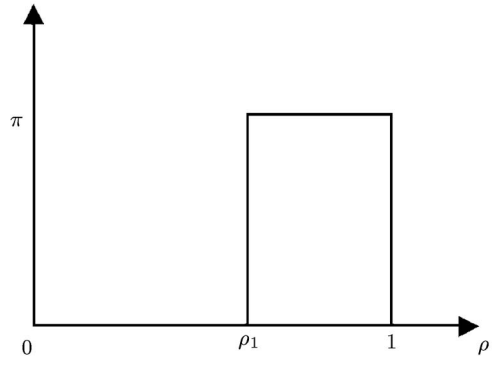


Fig. 1. Phase distribution of a filter with two zones.

Generally, the axial response $I(u)$ decreases as u increases. We introduce an important quantity $u_{1/2}$ that is defined as the defocus distance at which the intensity drops to one half of the in-focus intensity. It is a measurement of axial resolution. The smaller

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the value of $u_{1/2}$, the higher the axial resolution. The phase distribution of a filter with two zones phase-shifted by π is shown in Fig. 1. The zone boundary is defined by the normalized position ρ_1. This filter only modulates the phase of the pupil function.

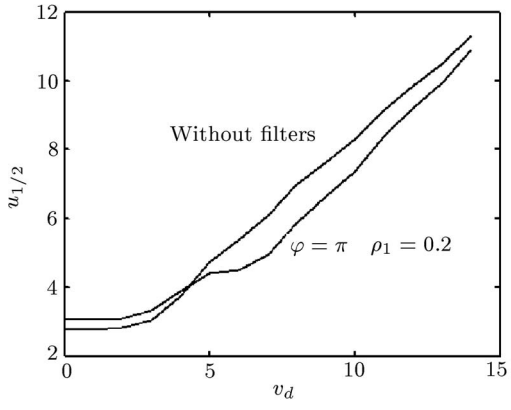


Fig. 2. Variations of $u_{1/2}$ with v_d.

Figure 2 shows the variations of $u_{1/2}$ as a function of v_d for a circular pupil and a phase-only filter ($\varphi = \pi$, $\rho_1 = 0.2$), respectively. When v_d is small, $u_{1/2}$ without filters is less than that with a filter. However, the situation is reversed if v_d is larger than a certain value. This value is 4.3 in the case of Fig. 2. The changes in $u_{1/2}$ as a function of ρ_1 for different v_d are illustrated in Fig. 3. Obviously, it can be seen that the optimum axial resolution for a given value of the detector radius can be obtained by altering the zone boundary of the filter. For example, the optimum ρ_1 is about 0.35, at which $u_{1/2}$ has the minimum value for $v_d = 7.45$.

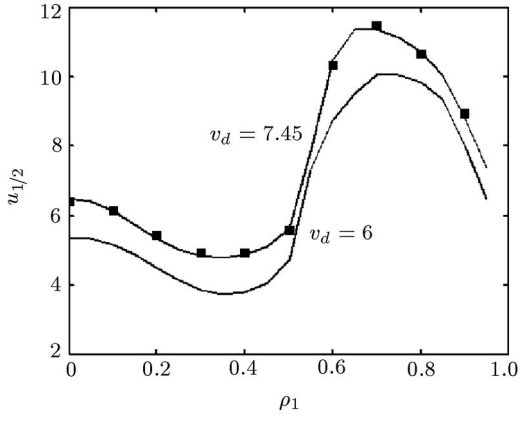


Fig. 3. Changes of $u_{1/2}$ with ρ_1 for different v_d.

In order to demonstrate the theoretical predictions, we have performed an experiment on an optical table. The experimental set-up is illustrated in Fig. 4. A He-Ne laser beam ($\lambda = 0.6328\,\mu$m) is focused by an objective O_1 on to a pinhole P_1 with a radius of 7.5 μm. The light from the pinhole P_1 is collimated by a lens L_1 with a focal length of 300 mm and then passes through a thin-film-transistor liquid crystal display (TFT-LCD). A $4f$ system is used to image the TFT-LCD on to the entrance pupil of a microscope objective O_2 (Olympus 10×, NA 0.25, Japan). The light reflected by a mirror M is finally focused on to another pinhole P_2 in front of the detector D, via an objective O_3 with a numerical aperture of 0.1. The mirror is controlled by a piezo driver (PI, Germany) and scanned along the axial direction.

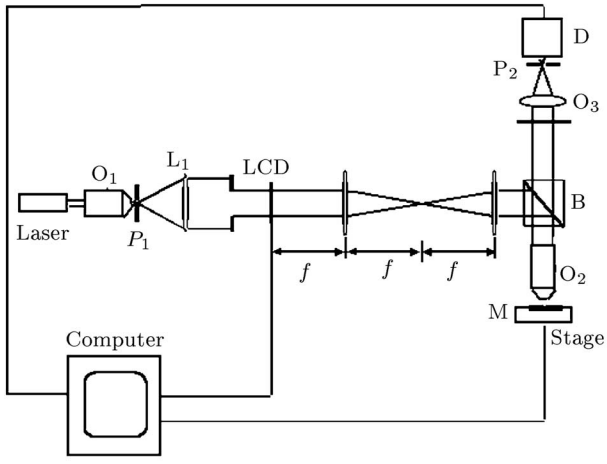


Fig. 4. Experimental set-up. B: beamsplitter, O: objective, P: pinhole, L: lens, LCD: liquid crystal display, M: mirror, D: detector.

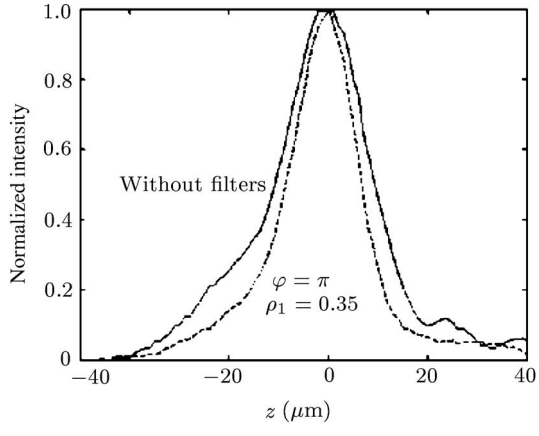


Fig. 5. Axial responses for $v_d = 7.45$.

In our case, we would obtain the phase modulation without any amplitude modulation. Thus, the polarizer behind the TFT-LCD is taken away. In this configuration, no amplitude modulation exists. The phase at the entrance pupil of the microscope objective O_2 is controlled by fending a voltage signal to each pixel of the display from a video card in a computer. This video card sends grey-level signals valued from 0 to 255. We have formulated a calibration look-up table of phase shift versus the grey level. In the experiments, the diameter of P_2 is set to be 15 μm, corresponding to the normalized detector radius of 7.45.

As mentioned above, for a given detector size, the axial resolution can be optimized. The experimental results are represented by the squares in Fig. 3. Typical normalized axial responses at $v_d = 7.45$ are shown in Fig. 5. The solid line is the axial response without filters, while the dashed line represents the optimum axial response when $\rho_1 = 0.35$. Obviously, the experimental results are in agreement with the theoretical predictions.

In summary, we have theoretically and experimentally shown that the axial resolution of a confocal microscope with a finite-sized detector can be improved by a filter with two zones phase-shifted by π, when the detector radius is larger than a certain value. Moreover, the axial response can be optimized by adjusting the zone boundary of the filter. This is of importance in the confocal microscopy, because almost all detectors are of finite size.

References

[1] Steve P 2002 *Science* **295** 1319
[2] Martínez C M, Pons A and Caballero M T 2002 *J. Opt. Soc. Am.* A **19** 1532
[3] Sales T R M and Morris G M 1997 *Opt. Lett.* **22** 582
[4] Boyer G 2002 *J. Opt. Soc. Am.* A **19** 584
[5] Juana D M D, Oti J E, Canales V F and Cagigal M P 2003 *Opt. Lett.* **28** 607
[6] Neil M A A, Juškaitis R, Wilson T and Laczik Z J 2000 *Opt. Lett.* **25** 245
[7] Gu M, Tannous T and Sheppard C J R 1996 *Opt. Lett.* **21** 312
[8] Ding Z H, Wang G Y, Gu M, Wang Z J and Fan Z F 1997 *Appl. Opt.* **36** 360
[9] Deng X Q, Liu L, Wang G Y and Xu Z Z 2000 *Optik* **111** 503
[10] Wilson T and Carlini A R 1987 *Opt. Lett.* **12** 227