Influence of Detector on Resolution in Confocal Imaging System*

Ge Huayong[1], Wang Baohua[2], Ren Qiushi[3]

1 *School of Information Science and Technology, Donghua University, Shanghai* 200051

2 *School of Communication and Information Engineering, Shanghai University, Shanghai* 200072

3 *Institute for Laser Medicine and Bio-photonics, Shanghai Jitong University, Shanghai* 200030

Abstract The imaging equations are derived based on point spread function, the influence of detector on resolution is simulated, and the optimum pinhole radius is proposed. To demonstrate the theoretical calculation, the confocal imaging system is designed and established. The axial response is measured for different pinhole, and the in-focus and out-of-focus images of bar target are obtained with the confocal system.

Keywords Confocal imaging; High resolution; Point spread function (PSF); Half width at half maximum (HWHM)

CLCN O438 **Document Code** A

0 Introduction

Confocal concept was proposed by M. Minsky in 1950 s[1] and wasn't fully developed until 1980 s[2~4]. In recent years, confocal imaging system has attracted much attention[5~9] since it provides a powerful means to achieve 3-D image of a thick object because of its optical-sectioning property. In principle, a point source is used to illuminate a point on the sample and the light emitted from this point is imaged through a pinhole placed at the proper position onto a photomultiplier tube (PMT). The major benefits of confocal system include superior resolution and contrast.

Most of the theory presented before on confocal system has taken the limiting case in which the pinhole is considered to be a point and hence represented mathematically by a Dirac delta function. In practice, however, the pinhole always has some finite size; otherwise it is impossible to detect any light[10~12]. For weak scattering object, the detected intensity is very small, so a large pinhole is also necessary to increase the output efficiency and the signal-to-noise ratio. In addition, its size plays an important role on the lateral and axial resolution of confocal system.

The detected image intensity was deduced, the influence of pinhole size on resolution was analyzed in this paper. A criterion for pinhole size is introduced to ensure confocal imaging and maximize signal-to-noise ratio, which is very helpful in the practical design of confocal imaging system. We set up a confocal system, measure the axial response with different pinhole and get the in-focus and out-of-focus images of bar target. The experimental results demonstrate the theoretical calculation and optical-sectioning capability of the confocal sytem.

1 Influence of detector on lateral resolution

In standard confocal imaging system, the detected image intensity of a point object with a finite detector can be expressed as[13,14]

$$I=|h_1|^2(|h_2|^2 * D) \tag{1}$$

where $*$ denotes the convolution operation, D is the sensitivity of the detector, h_1 and h_2 are the 3-D amplitude point spread function of the objective and the collector defined by

$$h_i(u,v)=\exp(\pm \mathrm{i} s_0 u)\int_0^1 P_i(\rho)\cdot \exp\left(\frac{1}{2}\mathrm{i}u\rho^2\right)\mathrm{J}_0(v\rho)\rho\mathrm{d}\rho \quad (i=1,2) \tag{2}$$

where $P_i(\rho)$ are the pupil function of the objective and collector, J_0 is a Bessel function of the first kind of zero order, u and v are optical coordinates related to radial and axial coordinates respectively, which are defined as

$$u=\frac{8\pi}{\lambda}z\sin^2\frac{\alpha}{2} \tag{3}$$

$$v=\frac{2\pi}{\lambda}r\sin\alpha \tag{4}$$

here, λ denotes the wavelength, $\sin\alpha$ is the numerical aperture.

For point detector, according to convolution theorem, the detected image intensity can be written as

$$I=|h_1(u,v)|^2|h_2(u,v)|^2 \tag{5}$$

while for infinite large detector, the detected image intensity should be expressed as

*Supported by National Natural Science Foundation of China(60371021)

Tel:021-50318160 Email:huayongge@yahoo.com.cn

Received date:2005-01-06

$$I=|h_1(u,v)|^2 \qquad (6)$$

The above equation shows that for infinite large detector, the objective determines the detected intensity and the collector has not any influence on it. In fact it becomes a conventional microscopy system when the pinhole size is larger than a certain size.

The parameter u in equation 2 represents the defocus of the lens. In our case we suppose the aberration-free lenses are focused on the object plane and detecting plane, and so we can set $u=0$. For simplicity, we assume that the objective and the collector are circular and of equal numerical aperture, also we suppose the finite size detector D is defined as $\mathrm{circ}(v/v_d)$, where v_d is the normalized radius of the pinhole. Under the above condition, the detected image intensity as a function of detecting position for a variety of pinhole size can be expressed as

$$I(v,v_d)=\left[\int_0^1\int_0^1 J_0(v\rho)J_0(vt)\rho t\,\mathrm{d}\rho\,\mathrm{d}t\right]\cdot \int_{v-v_d}^{v}\int_0^1\int_0^1 J_0(\rho t)J_0(mt)\rho m\,\mathrm{d}\rho\,\mathrm{d}m\,\mathrm{d}t \qquad (7)$$

which is plotted in Fig. 1 for a variety of pinhole radius v_d. It is clear that the smaller the pinhole is, the sharper the peak is. Namely the confocal system has narrower HWHM for smaller pinhole, which can also be found in Fig. 2. It shows the variation of the HWHM $v_{1/2}$ of the curves of Fig. 1 as a function of v_d. We notice that the HWHM

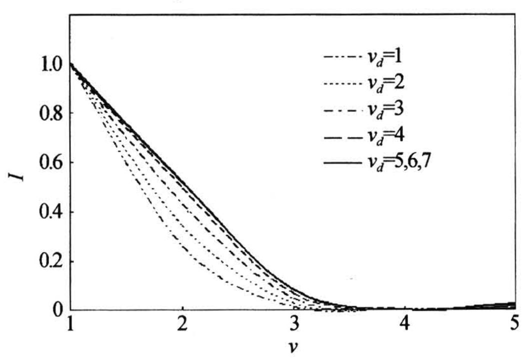


Fig. 1 The variation of normalized intensity

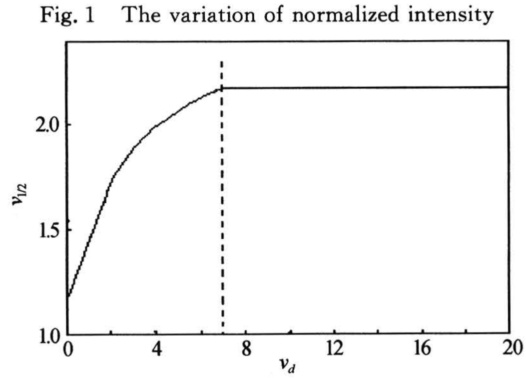


Fig. 2 HWHM $v_{1/2}$ of the lateral response intensity

rises with the increase of pinhole size and remains constant for $v_d > 7$, which corresponds to the indistinguishable curves in Fig. 1. Therefore when $v_d>7$, all the in-focus and out-of-focus signal can pass the pinhole and then be detected by PMT. It is actually not a confocal imaging system but a conventional microscopy system.

2 Influence of detector on axial resolution

The other important advantage of confocal system is the depth-discrimination property. Under the above condition, the axial response as a function of the distance from focal plane in standard confocal system may be written as

$$I(u,v_d)=\int_0^{v_d}\left|\int_0^1 \exp(iu\rho^2)J_0(v\rho)\rho\,\mathrm{d}\rho\right|^2 v\,\mathrm{d}v \qquad (8)$$

It can be seen clearly from the above equation that the detected intensity is related with the pinhole size. Fig. 3 shows the HWHM $u_{1/2}$ of axial response intensity as a function of v_d. Here, $v_d=0$ corresponds to point detector. With the increase of v_d, the HWHM becomes wider and axial resolution lower. We also notice that the HWHM remains nearly constant for $v_d<3$ and increases rapidly with the rise of pinhole size for $v_d>3$.

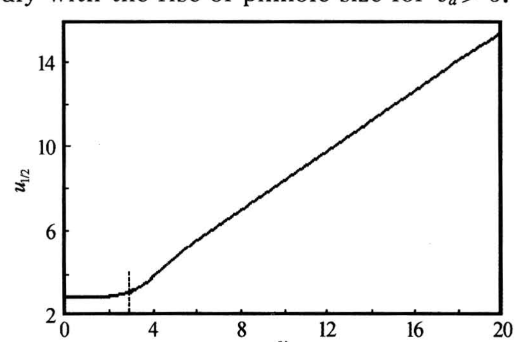


Fig. 3 HWHM $u_{1/2}$ of the axial response intensity

3 Experiment and discussion

To test the theoretical calculation, we set up a confocal imaging system which is shown in Fig. 4.

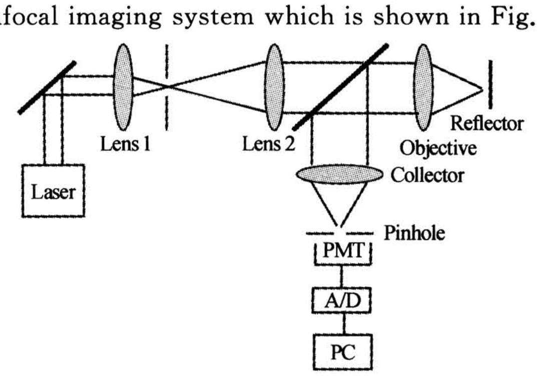


Fig. 4 The confocal system constructed

In the system a He-Ne laser operating at 632.8 nm was used as the illumination light. Lens 1 and lens 2 were used to expand the beam. Two 10× lenses were used as collector and objective. A PMT was used as the detector and a reflector was used as the sample.

The beam from He-Ne laser passes through the illumination path and focuses on the sample. The reflected signal from sample passes through detecting path and received by PMT. When the reflector is put on the focal plane, we can get the maximum signal. However, when the reflector is away from the focal plane, the detected signal decreases. By moving the reflector axially, the axial response of the confocal system can be obtained.

Fig. 5 shows the axial response for different pinhole radius. Obviously, larger pinhole brings wider axial response, therefore lower axial resolution. The experimental results are in accordance with theoretical analysis. Fig. 6(a) and Fig. 6 (b) show the in-focus and out-of-focus images of bar target, respectively. It can be seen from the figures that the in-focus image is more clearer and has higher contrast than out-of-focus image. Therefore depth-discrimination (optical sectioning) capability can be achieved by the confocal imaging system constructed.

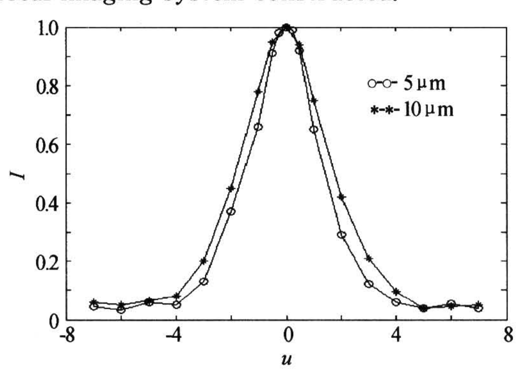
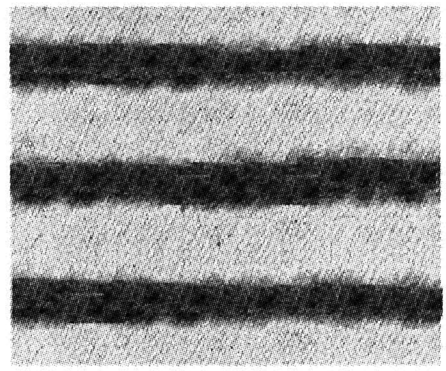
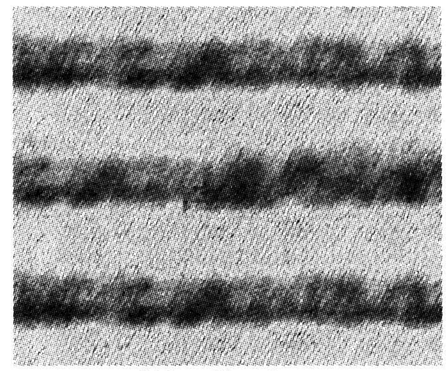


Fig. 5 Axial response with different pinhole

According to the above analysis, the pinhole size has great influence on lateral and axial resolution. In order to achieve a true confocal image, the size of the pinhole should be chosen as small as possible. In practice, however, the pinhole always has some finite size; otherwise it would not be possible to detect any light. So a larger pinhole is also necessary to increase the output efficiency and signal-to-noise ratio especially for weak scattering object. According to Fig. 2 and Fig. 3, $v_d=3$ may be a reasonable choice of pinhole size that can ensure confocal imaging and also maximize signal - to - noise ratio . As an



(a)In=focus image



(b)Out-of-focus image

Fig. 6 Images obtained with the confocal imaging system

example, for a 20×, 1.5-numerical-aperture lens, this criterion suggests that a 4.0 μm-radius pinhole is needed when He-Ne laser operating at 632.8 nm is used.

4 Conclusion

We derive the lateral and axial image intensity distribution and analyze the influence of pinhole size on the resolution. Our research shows that the confocal imaging system has narrower HWHM and higher resolution for smaller pinhole. To demonstrate the theoretical calculation, we set up a confocal system, measure the axial response of the system and obtain the in-focus and out-of-focus images of bar target. At last, we also introduce a criterion for pinhole size to ensure confocal imaging and maximize signal-to-noise ratio, which is very useful in the practical design of confocal system.

Reference

1 Minsky M. Microscopy apparatus. USA Patent, 30133467. 1961

2 Wilson T. Confocal microscopy. London: Academic Press, 1990. 1~78, 123~156

3 Gu M. Principles of three-dimensional imaging in confocal microscopes. Singapore: World Scientific Publishing Co. Pte. Ltd, 1996. 1~174

4 Wilson T, Sheppard C J R. Imaging and super-resolution in the harmonic microscope. *Opt Acta*, 1979, **26**(8): 761 ~770

5 Gu M, Sheppard C J R. Three-demensional coherent

transfer functions for confocal imaging with unequal annular lenses. *J of Modern Opt*, 1993, **40**(7): 1255～1272

6 Yang C P, Tang Z L, Pei H J. Imaging analysis of two-photon confocal microscopy with different fluorescence wavelength. *Acta Photonica Sinica*, 2004, **33**(2): 159～163

7 Gao W R, Tao C K, ang X C. Relationship between the two dimensional resolution and signal-to-noise ratio of laser confocal microscopy. *Acta Photonica Sinica*, 2002, **31**(6): 730～735

8 Qi Y J, Chi Z Y, Chen W J, *et al*. The further studying of PSF in confocal scanning microscopy system. *Acta Photonica Sinica*, 2002, **31**(8): 1029～1031

9 Fu L, Luo Q M, Lu Q, *et al*. Effect of sattering and absorption properties of bidogical tissue on two-photon excitation and confocal imaging depth. *Acta Photonica Sinica*, 2001, **30**(3): 262～266

10 Wilson T, Carlini A R. Size of the detector in confocal imaging system. *Opt Lett*, 1987, **12**(4): 227～229

11 Gu M, Sheppard C J R. Three-dimensional partially-coherent image formation in confocal microscopes with a finite-sized detector. *J of Modern Opt*, 1994, **41**(9): 1701～1715

12 Drazic V. Three-dimensional transfer function analysis of a confocal fluorescence microscope with a finite-sized source and detector. *J of Modern Opt*, 1993, **40**(5): 879～887

13 Wilson T, Sheppard C R J. Theory and practice of scanning optical microscopy. London: Academic Press, 1984. 42～78

14 Sheppard C J R, Wilson T. Image formation in scanning microscopes with partially coherent source and detector. *Optica Acta*, 1978, **25**(4): 315～325

共聚焦成像系统中探测器对分辨率的影响

葛华勇[1] 王保华[2] 任秋实[3]

(1 东华大学信息科学与技术学院，上海 200051)

(2 上海大学通信与信息工程学院，上海 200072)

(3 上海交通大学激光与光子生物研究所，上海 200030)

收稿日期：2005-01-06

摘 要 基于点扩展函数，推导出共焦系统的成像公式，模拟了探测器尺寸对分辨率的影响，并提出最佳针孔半径. 为验证理论分析，设计并建立了一套共焦系统，测量了不同针孔时的轴向响应，并得到了鉴别率板的共焦和离焦像.

关键词 共聚焦成像；高分辨率；点扩展函数；半高宽

Ge Huayong was born in 1976. She graduated from physics major in 1999, received the M. S. degree in 2002 from Shanghai University, and received the Ph. D in June in 2005 from Shanghai University.