

# The calibration of tilting and refractive index of specimen in confocal scanning microscopy

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## Abstract

Confocal scanning microscopy(CSM) has been reported as an excellent method using the optical probe in scanning probe microscopy. However, it has the problems as formerly which make allowance for several off-axis effects from the deflection of beam or high numerical aperture, the lateral shift of primary focal point and the conditions of specimen. These give rise to the poor resolution as the low spatial frequency and the obstacle of design the unitary lens and the other optical components. The axial distortion is resulted from the increase of the nonlinearity of optical transfer function. This article shows these primary problems in the image reconstruction. This paper shows the response experimently when the error sources such as the spatial offset of the elements change and the parasitic condition of specimen, and the mathematical expression of the light in the optical fields of system. And we propose the progressive bounds of the geometry of optical components.

Keywords : confocal scanning microscopy(CSM), the lateral shift of primary focal point, optical transfer function, the parasitic condition of specimen , optical fields

## 1. Introduction

CSM(confocal scanning microscopy) has been used in biomedical application, materials science, semiconductor quality control and forensic application[1]. A confocal scanning microscope consists of a light projection device with which a small spot of a light beam is shaped on a specimen, a light detector device with a pinhole or single mode fibre[2], and a mechanism for two-dimensional beam scanning. An image profile can be regenerated in confocal scanning microscopy with many advantages of non-contact, high speed, high resolution of thick specimen comparatively. But it is an important matter for high resolution to align pinhole and other optical components.

Generally, the conditions of specimen give rise to the fatal distortion of an image profile, for instance, the tilting on the automated z-axis stage to section optical image[3] and variable refractive index. In a practical case, the peak intensity of detecting signal in the confocal point spread function diminishes by a factor of two or more when the beam spot is focused with less than 10 micron meter onto the specimen according to variation of refraction index[4].

In addition to the axial movement of the primary focal point having the information of specimen, the lateral movement of it gives a necessity of the unitary lens to the scanning system. Using the scanning mirror, the pupil of beam should be fixed at the nominal position. Further more, the use of a scanning mirror may result

in a reduced numerical aperture of the objective lens and the loss of a great proportion of the radiation[5].

## 2. System description

In addition to conventional confocal microscope, two-dimensional scanner is used as shown at fig.1. Acousto optical deflector has advantages as variableness, speed and position controllability but disadvantages as dispersiveness, poor efficiency deflection dependent and complication optical set-up[5]. Most important fact is that the scanning speed of the object scanning type depends on the dynamic of automated XY stage almost. Technical progresses in the past have led to scanners with greater accuracy and higher frequencies of operation at larger scan angles with wider apertures. This system is used with the bulk acousto-optic deflector, which has the center drive frequency of 100 MHz and the active aperture of 2.5 mm for itself. In the separation of deflector and dichroic mirror, the unitary lens is used for the reduction of loss of energy.

## 3. Wave form through optical path

The source of beam is HeNe laser suffered from the uncertainty of wavelength. Neglecting it, through the filtering

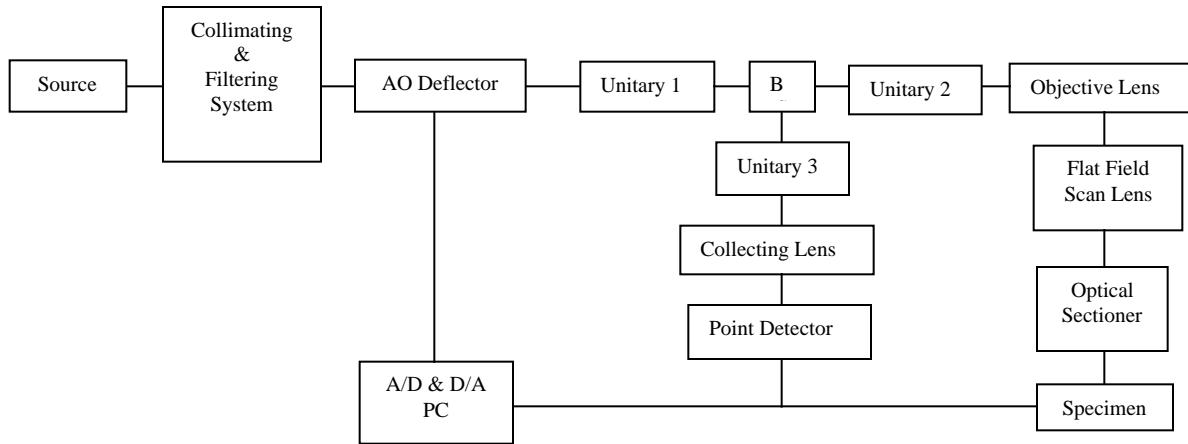


fig.1 Function scheme of confocal scanning microscopy using AO

and collimating system including lenses and pinhole, the wave leads to the diffractive light because of the finite size. And at the AO deflector, Fresnel diffraction[8] appears. After the AO deflector, the deflection angle of the diffracted beam can be expressed as,

$$\theta = \sin^{-1} \frac{\lambda_{of}}{2n_d \lambda} [1 - (\frac{\lambda}{\lambda_{of}})^2 (n_i^2 - n_d^2)] \quad (1)$$

where  $\lambda_{of}$  is the free space wavelength of the incident light,  $n_i$  and  $n_d$  are the indices for the incident and diffracted wave respectively[6].

In conventional confocal microscopy, the predominant approximations are used, if the lens of high numerical aperture is not involved. They are such conditions in which we have thin lenses and first order theory is sufficient for their analysis. Fourier analysis leads to a marvelous way of treating optical processes in terms of spatial frequency [6].

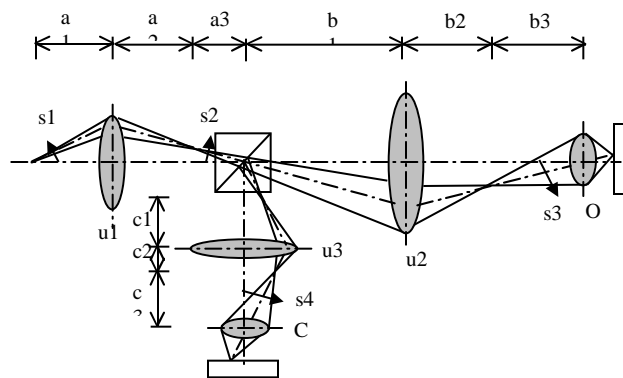


fig.2 The primary optical system in scanning microscopy

The optical component including common objects, pinhole, lenses, film, scanner and other detector has a finite pupil function in practice. From Maxwell's equations[7], a vectorial form of the wave equation for the propagation of light can be obtained. The Kirchhoff diffraction formula is a solution to the Helmholtz equation that is derived a scalar form of the wave equation neglecting the polarization properties of light. The diffracted light in the condition can be expressed as

$$2 = \frac{i}{\lambda} \cos(n,r) \quad {}_1(x_1, y_1) \frac{\exp(-ikr)}{r} dx_1 dy_1 \quad (2)$$

where  ${}_1$  is the light field before the diffraction element,  ${}_2$  after the element,  $r$  is the distance between the two diffraction plane,  $k$  is the wave number and  $\cos(n,r)$  denotes the cosine of the angle between the wave vectors. The angle factor can be an important one in the scanning condition. The spherical wavelet propagator is usually expressed as the series including the coupling coordinates, which leads to Fourier transform of the combining wavelet.

The geometry of the optical system can be expressed as fig.2. Beam Scanning suffer from serious problem if the microscope is to operate at highest resolution [5]. If light is deflected by a deflector and is incident on an objective then either the full aperture of the lens will not be properly filled at all times during the scan, resulting in the objective having a reduced numerical aperture and a consequent loss of resolution or alternatively a great proportion of the radiation will be lost. In addition to primary lens, some lens system may be needed in order to minimize the loss of energy of

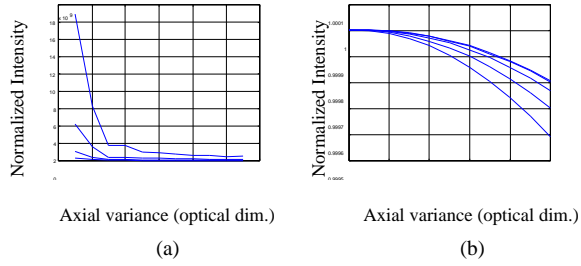


fig.3. The axial variance of the focal plane vs intensity of detection signal at zero deflection (a) when NA of Objective lens increases from 0.1 to 0.4, (b) when the finite size of pinhole increases from 20 to 60 micron meter.

light. In fig.2, the entrance pupil of beam can be fixed at the beam splitter, objective lens and collecting lens where the incident lights may be crossed twice.

Fig.3 (a) denotes that the high numerical aperture of the objective or collecting lens leads to the good sensitivity and the large nonlinearity as aberration. That means that the intensity profile for the open loop scanning method should be matched with its response to a perfect mirror as specimen, which can be minimized with the optical sectioning or the optical probe of the closed loop control. fig.3 (b) show that the axial response is a function of the finite size of detector. For a point detector, in which case imaging is coherent, the axial response becomes independent of the defocus distance in the limiting case of an infinitely large detector. The sensitivity of response increases greatly when the detector size decreases. The alignment of optical components becomes, however, a critical issue.

#### 4. Ray path & design parameters

When the beam is scanned, the off-axis effects distort the waveform at each plane. In practice, it is important to know how seriously lens aberrations or defocus affect the optical performance of the microscope. At first, the optical axis should be traced as shown at the dashed line of fig.2. The incident angle at the beam splitter can be expressed as,

$$\theta_2 = \tan^{-1} \frac{a_1 \tan \theta_1}{a_2 + a_3} \quad (3)$$

at the objective lens as,

$$\theta_3 = \tan^{-1} \frac{b_1 \tan \theta_2}{b_2 + b_3} \quad (4)$$

and the collecting lens as,

$$\theta_4 = \tan^{-1} \frac{(a_3 + c_1) \tan \theta_2}{c_2 + c_3} \quad (5)$$

Three-dimensional transfer functions are based on the paraxial approximation. As this assumption may not hold for an objective of a numerical aperture higher than  $1/\sqrt{2}$  we use the lenses of 0.25 NA. In the second

place, the lateral shift of light should be defined at the specimen and point detector. When the collimated light shows the deflected path at each elements, the remain parameters can be expressed as,

$$a_3 = \frac{f_{u1}^2}{a_1 - f_{u1}} \quad (6)$$

$$b_3 = \frac{\{(a_3 + b_1) f_{u3}\}^2}{a_1(a_3 + b_1 - f_{u2})^2 + (a_3 + b_1 - f_{u2})(a_3 + b_1) f_{u2}} \quad (7)$$

$$c_3 = \frac{(c_1 f_{u3})^2}{(c_1 + a_3)(c_1 - f_{u3})^2 - (c_1 f_{u3})^2 (c_1 - f_{u3})} \quad (8)$$

where  $f_{ui}$  is the focal length of each unitary lens. Then the lateral shift of light at  $L_s$  the specimen is,

$$L_s = \frac{f_o b_3}{b_3 - f_o} \tan \theta_3 \quad (9)$$

and the lateral shift of light at the  $L_d$  detector is,

$$L_d = \frac{f_c c_3}{c_3 - f_c} \tan \theta_4 \quad (10)$$

where  $f_o, f_c$  is the focal length of objective, collecting lens respectively. The important facts can be explained with the results. All parameters must be positive number in the reasonable limits and three parameters for the design should be chosen in order to minimize the cost function which can be expressed as,

$$M = W_s S_s L_s + \frac{W_d}{S_d L_d} \quad (11)$$

where  $W_s, W_d$  is the weights and  $S_s, S_d$  is the scale factor of beam shift at the specimen and detector respectively. Of course,  $L_d$  is a function of  $a_1, b_1$  and  $L_s$  of  $a_1, c_1$ . If the design parameters and weights are chosen properly in order to minimize the cost function  $M$ , the optimal condition can be acquired. When  $a_1, b_1$  is changed in the reasonable bound, the lateral shift at the specimen (the scanning range) can be shown as fig.4 (a). At the same time, the lateral shift of light at the detector like as pinhole changes for the variance of  $c_1$ . At the results, we can find the design variables of the optical system.

#### 5. The condition of specimen

AO deflector has n-th order ray as multi-beam. Further more, the multi-beam is a symmetric form about optical

axis. This fact is the method of not only twice-stroke scanning, but also compensation for the condition of specimen. The signals of 1<sup>st</sup> and -1<sup>st</sup> order may have the low frequency distortion, the waviness of detection, because of the tilt of specimen or curvature of field. These problems can be removed by concerning the signals, for instance, the summing or extraction of normalized mean values.

## 6. Experiments

After choosing the design parameters for the cost function of other variables, the tolerance of set-up is a dominant problem. At fig.5, 'H' shape height sample with 80nm height and 2 micron meter pitch is scanned. If the amount of lateral beam shift at the detector is large comparing the size of pinhole, the waviness of signal possessing the information of specimen is evident, fig.5 (a)(b). In this case, the effect of beam shift at the pinhole is more fatal than the curvature of field. However, if the design parameters is given properly in order to minimize the later shift of light at the detector, the waviness can be eliminated dramatically, fig.5(c).



fig.4. The lateral shift of light with the variance of a1 (a) at the specimen when b1 increases from 45 to 60mm, (b) at the point detector when c1 increases from 135 to 150 mm.

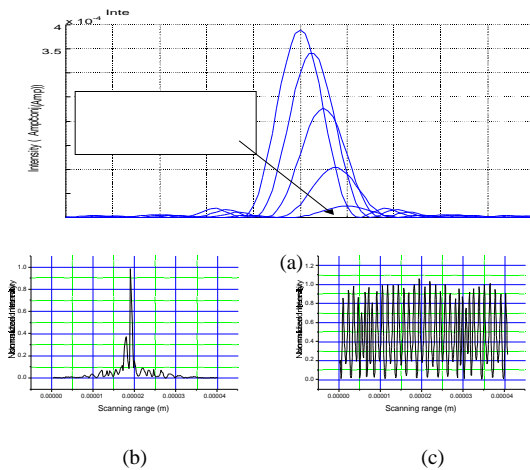


fig.5. 'H' shape height sample with 80nm heigh and 2 mu meter pitch, (a) the effect of beam shift at the pinhole, (b) the scanning result having the waviness, (c) with the design parameters using minimization of beam shift and compensation for the condition of specimen.

## 6. Conclusion

Confocal scanning microscopy (CSM) has the problems as which make allowance for the lateral shift of primary focal point. These give rise to the poor resolution as the low spatial frequency and the obstacles of design the unitary lens and the other optical components. We propose the progressive bounds of the geometry of optical components with the cost function having the scanning range and lateral shift of beam at the detector.

## 7. Acknowledgement

This work was supported by IITA on Korea under grant C1-98-0110-00. The author thanks IITA for their financial support.

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