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DETECTOR ARRAYS IN CONFOCAL SCANNING MICROSCOPES

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Abstract

We consider the use of detector arrays in scanning microscopes and show that confocal operation may be achieved in a variety of ways. We base our analysis on a consideration of the form of the optical transfer function or transmission cross coefficient. This reveals that the Fourier transform of the detector sensitivity function is the function of importance. It is not necessary that this function be constant over the whole space and hence a variety of detector sensitivity functions will give fully confocal imaging. The traditional method of a limiting point-like detector is a special case, but one which has advantages in the rejection of scattered and flare light from the image.

Key Words: Optical Microscopy, Scanning Microscopy, Image Formation, optical sectioning, detector geometry, detector size, noise, flare light, pupil function, confocal imaging.

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Introduction

The only difference between conventional scanning microscope and a confocal one lies in the form of the detector [Wilson and Sheppard, 1984]. The conventional arrangment uses a large area detector whereas the confocal employs a point like detector. The development and commercial availability of photodetector arrays cause us to re-examine the role of the detector geometry. In this paper we will consider a system employing a point source of light, but with a detector of arbitrary intensity sensitivity, D, Figure 1. We shall begin by describing the optical image formation in terms of an optical transfer function and discuss how the form of the detector modifies this function and hence the spatial coherence of the imaging. We will then go on to discuss the optical sectioning properties of systems with arbitrary detector sensitivity functions and show that the traditional method of realising a confocal microscope is just a special case of a more general distribution function.

Theoretical Considerations

We consider the geometry of Figure 1 and assume that the object is sufficiently thin that it may be described by an amplitude transmittance (or reflectance) t(x,y).

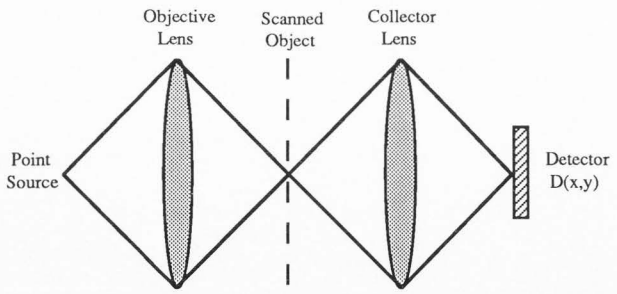

Fig.1. The optical system of a scanning microscope consisting of a point source and a large area incoherent detector of arbitrary intensity sensitivity, D.

We introduce the Fourier transform (or spectrum) of this object, T(m,n) via:

$$T(m, n) = \int_{-\infty}^{+\infty}\int t(x, y)\exp - 2\pi j(mx + ny)dxdy \qquad (1)$$

and

$$T^*(p, q) = \int_{-\infty}^{+\infty}\int t^*(x, y)\exp 2\pi j(px + qy)dxdy \qquad (2)$$

where m, n, p and q are spatial frequencies and the asterisk denotes the complex conjugate. We can now write the image intensity as [Sheppard and Wilson, 1978]:

$$I(x_s, y_s) = \int_{-\infty}^{+\infty}\int\int\int C(m, n; p, q)T(m, n)T^*(p, q)$$
$$\exp 2\pi j\{(m - p)x_s + (n - q)y_s\}dmdndpdq \qquad (3)$$

where C(m,n;p,q) is the partially coherent transfer function or transmission cross coefficient. It depends only on the form of the optical system. If we assume that the lenses in Figure 1 have pupil functions $P(\xi,\eta)$ where (ξ,η) are variables in the pupil plane and that the detector sensitivity, D(x,y) has a Fourier transform, F_D, given by:

$$F_D(m, n) = \int_{-\infty}^{+\infty}\int D(x, y)\exp - 2\pi j(mx + ny)dxdy \qquad (4)$$

then, for a unity magnification system, we can write:

$$C(m, n; p, q) = \int_{-\infty}^{+\infty}\int\int\int P(\xi_1, \eta_1)P^*(\xi_1^1, \eta_1^1)P(\lambda fm - \xi_1, \lambda fn - \eta_1)$$
$$P^*(\lambda fp - \xi_1^1, \lambda fq - \eta_1^1)F_D\left[\frac{\xi_1^1 - \xi_1}{\lambda f}, \frac{\eta_1^1 - \eta_1}{\lambda f}\right]$$
$$d\xi_1\, d\eta_1\, d\xi_1^1\, d\eta_1^1 \qquad (5)$$

where λ is the wavelength and f is the distance between the objective and the object. The traditional confocal case arises when $F_D = 1$ and the conventional when $F_D(\alpha,\beta)=\delta(\alpha)\delta(\beta)$ where $\delta(-)$ is a Dirac delta function and α, β are dummy variables. The fact that $F_D = 1$ in traditional confocal microscopy is overly restrictive as the pupil functions P are themselves only non-zero over a limited range. Our basic contention, which we will explore further, is that in order to achieve confocal operation we need F_D to be unity only over the space in which the product of the pupil functions in equation (5) is non-zero.

Let us now consider the image of some specific objects. We begin with a point object, for which we can write:

$$I(t, w) = |h(v)|^2\left\{|h(t, w)|^2 \otimes D(t, w)\right\} \qquad (6)$$

where $v^2 = t^2 + w^2$, the symbol $\otimes$ denotes the convolution operation and t, w are normalised optical co-ordinates, related to real distance x and y via relationships like:

$$t = \frac{2\pi}{\lambda} \,.\, x \,.\, \sin\alpha \qquad (7)$$

where $\sin\alpha$ is the numerical aperture. The function h is the amplitude point spread function of the lens, given by the Fourier transform of its pupil function.

We have chosen to write equation (6) in a form using t and w to emphasise that, because h is circularly symmetric, the image of a point object will not, itself, be circularly symmetric unless D is also circularly symmetric. As an example, if a slit shaped detector is used an asymmetric image results [Wilson and Hewlett, 1990]. We will concentrate on circularly symmetric detectors in the following. In doing this we need to recall the mathematical relationship that:

$$a \otimes b = a \qquad (8)$$

provided the Fourier transform of b is constant (unity) over the extent of the Fourier transform of a. This implies that we can write equation (6) as:

$$I(v) = |h(v)|^4 \qquad (9)$$

provided that the Fourier transform of D is constant over the extent of the Fourier transform of $|h(v)|^2$. It is usual to define the pupil functions as:

$$P(\rho) = \exp\frac{1}{2}ju\rho^2 \qquad |\rho| \le |$$
$$= 0 \qquad \text{otherwise} \qquad (10)$$

where the term in u denotes the degree of defocus and ρ is a normalised radial coordinate in the pupil plane. We will return to this term later. In the absence of defocus, (u=0), equation (9) becomes:

$$I(v) = \left(\frac{2J_1(v)}{v}\right)^4 \qquad (11)$$

where J_1 is a first order Bessel function of the first kind.

It is clear that the Fourier transform of $|h|^2$ has an extent to 2.0 normalised units. This suggests that if the detector sensitivity were, say:

$$D(v) = \frac{2J_1(av)}{av} \qquad (12)$$

then as long as $a \ge 2$ the image of a point object would be given by equations (9) and (11). We note that $a \to 0$ gives D(v) = constant (conventional operation) and $a \to \infty$ gives $D(v) = \delta(v)$ (traditional confocal operation).

One of the main advantages of confocal microscopy concerns the ability to reject detail outside the focal plane. A useful metric of this optical sectioning property is to consider the signal as a perfect reflector is scanned axially through focus. If we model this object via t(x,y) = 1 or $T(m,n) = \delta(m)\delta(n)$ we find from equation (3) that:

$$I(u) = C(0, 0; 0, 0) \qquad (13)$$

and

$$C(0, 0; 0, 0) = \int_{-\infty}^{+\infty}\int\int\int F_D\left(\xi_1 - \xi_1^1, \eta_1 - \eta_1^1\right)P^2(\xi_1, \eta_1)$$
$$P^{*2}\left(\xi_1^1, \eta_1^1\right)d\xi_1\, d\eta_1\, d\xi_1^1\, d\eta_1^1 \qquad (14)$$

when we have assumed that the pupil functions are even functions.

Again we need F_D to be constant over the region where the product of the pupil functions are non-zero. We can recast equation (14) as:

$$I(u) = \int_{-\infty}^{\infty} \left| h(2u, v) \right|^2 D(v) v dv \qquad (15)$$

which can be thought of as a special case of a convolution when the convolution variable is zero. The condition is the same as we discussed previously. If we can take $a \geq 2$ in our detector of equation (12) then:

$$I(u) = |h(2u, 0)|^2 \qquad (16)$$

or [Wilson and Sheppard, 1984]:

$$I(u) = \left(\frac{\sin u/2}{u/2} \right)^2 \qquad (17)$$

where u is related to real axial distance z via:

$$u = \frac{8\pi}{\lambda} . z . \sin^2 \alpha/2 \qquad (18)$$

We plot, in Figure 2, the optical sectioning as given by equation (15) for the case of the Bessel function shaped detector. As we have already said any value of $a \geq 2$ gives ideal confocal behaviour and hence values of $a < 2$ give correspondingly poor sectioning until the effect disappears altogether when $a = 0$. The latter case corresponds, of course, to the conventional microscope.

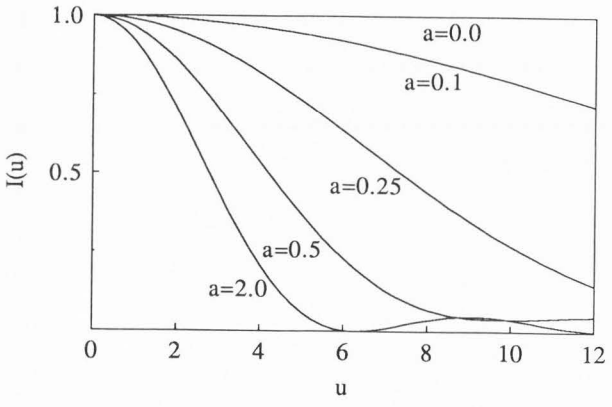

Fig.2. The variation of I(u) against u for a variety of Bessel function detector sensitivities. Note that for all values of $a \geq 2$ that I(u) is given by equation (17).

A property of confocal systems is that the imaging is coherent such that we can write $C(m;n;p,q) = c(m,n)c^*(p,q)$. It is clear from the geometry of equation (5) that this is achieved for our Bessel function detector if $a \geq 2$.

Noise

One of the main side results of confocal microscopy is that the use of a limiting pinhole-type aperture serves to reduce the amount of flare and scattered light present in an image. In an attempt to quantify these effects we consider a transmitted light system with no object, but with a detector sensitivity given by equation (12). This permits us to write the detected signal as:

$$I_{det} = \int_0^{\infty} \left(\frac{2J_1(v)}{v} \right)^2 \frac{2J_1(av)}{av} . vdv \qquad (19)$$

which is, as we have seen before, constant for $a \geq 2$. If we further assume that the intensity of the flare and scattered light is simply proportional to the area of the detector we can write:

$$I_{flare} = \int_0^{\infty} \frac{2J_1(av)}{av} vdv \qquad (20)$$

which is inversely proportional to a^2. This permits us to derive an expression for signal to flare ratio as a function of a. It is clear that although confocal operation may be achieved for $a \geq 2$ that the signal to flare ratio is considerably enhanced if a is made as large as possible. Indeed the ratio is proportional to a^{-2} for $a \geq 2$. We recall that $a \rightarrow \infty$ reverts to the case of a traditional point confocal detector.

Conclusions

We have discussed the image formation in scanning microscopes with arbitrary detector sensitivity functions and have shown that the traditional method of achieving confocal operation is merely a very special case although one which has advantages from the point of view of signal to noise ratio. The case of fluorescence confocal microcsopy may be treated similarly. Here it is also found that a detector of the form of equation (12) will give confocal operation. However in this case the value of a may be scaled by the ratio of the fluorescent to excitation radiation wavelengths.

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Discussion with Reviewers

R.W. Wijnaendts van Resandt: You have shown that a variation of the detector geometry and sensitivity can be used to optimise the system response function. Is it in principle possible using a detector array to extract both phase and amplitude of a transmission confocal microscope?

Author: I think it would be difficult to extract phase information with a detector placed as shown in Figure 1. It is however possible, by the use of suitable beam splitters and infinity tube length objectives, to place a detector array at an equivalent position to the pupil of the second lens. It is then easy to obtain differential phase contrast information by using the array to mimic a quadrant detector. In this way phase and amplitude information can be obtained simultaneously.

V.K. Chen: Would you comment on how optical sectioning or depth response is changed by detector shape and detector sensitivity distribution?

Author: The general rule is that the smaller the detector the better is the sectioning. It is also true that the detector should ideally be circular in order to image all object features equally. An obvious attraction of a detector array is that we may mimic the following situation. Circular detectors of two different radii have two different sectioning strengths. If we subtract a fraction of the signal from one detector from the signal from the other detector we can tune the sectioning to be arbitrarily sharp. The price we pay is that the I(u) function may go negative for certain values of u. In practice with an array this could be achieved with a central circular detector together with a surrounding annular ring. Of course, the larger the detector the more noise related problems we are likely to run into.

A further attraction of a line or slit shaped detector array lies in the speed of image acquisition in that we need only scan the object or light beam in one direction. The sectioning properties with slit detectors whilst not as good as true confocal point detectors are still quite acceptable for many applications. The asymmetry in the image in the case of a slit detector is found to be far less pronounced in the case of fluorescence imaging. The asymmetry becomes more noticeable as the difference between excitation and fluorescence wavelengths becomes greater.