Scanning Microscopy

Volume 3 | Number 1 Article 2

11-14-1988

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T. Wilson
Oxford

Scanning Microscopy, Vol. 3, No. 1, 1989 (Pages 9-13) 0891-7035/89$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

OPTICAL DESIGN CONSIDERATIONS IN CONFOCAL SCANNING MICROSCOPY

T. Wilson

Department of Engineering Science
Parks Road,
Oxford.
OX1 3PJ
England

(Received for publication May 25, 1988, and in revised form November 14, 1988)

Abstract

We review the properties and applications of confocal optical systems. We concentrate on non-fluorescent reflection systems and on applications which make use of the unique optical sectioning property of these instruments to provide three-dimensional images. A crucial design choice concerns the form and size of the detector. We therefore concentrate on the role of the detector size on the strength of the optical sectioning. We find that although the sectioning becomes weaker as the detector sizes where the sectioning remains essentially constant. We also consider the role of lens aberrations which are inevitably present and find, inter alia, that these reduce the strength of the sectioning.

Key words: scanning microscopy, confocal microscopy, optical sectioning, detector geometry, detector size, lens aberrations, three-dimensional imaging, noise, flare light, pupil function.

Address for Correspondence:
Department of Engineering Science, University of Oxford, Parks Road, OXFORD, OX1 3PJ, England.
Phone No: (0865) 273000

Introduction

A scanning optical microscope can be built which has imaging properties identical to those of conventional microscopes [Wilson and Sheppard, 1984]. It also, however, has several specific advantages over the conventional instrument in that the image is obtained in a serial electrical form and so is ideal for subsequent computer processing and display. The beauty of the scanning approach is that we relax the requirement that the optical system should be able to image the whole object field at the same time. In the scanning microscope we merely ask the optics to image one picture point: the whole field is then built up by scanning. This decoupling of the magnification (which is determined by the scanning) from the resolution (which is determined by the optics) permits certain modifications to the optical system which are not possible in conventional optical microscopy. The confocal optical system is one such modification [Minsky, 1957, Wilson and Sheppard, 1984].

Figure 1 shows a schematic of a confocal microscope system. The only difference between this system and a conventional microscope is the use of a point detector (often a small pinhole placed in front of a photodiode or photomultiplier tube). The physics of confocal image formation have been discussed in great detail elsewhere (see e.g., Wilson and Sheppard, 1984) and so we will merely summarise the key points here. The presence of the point detector ensures that both lenses contribute equally to the image formation.

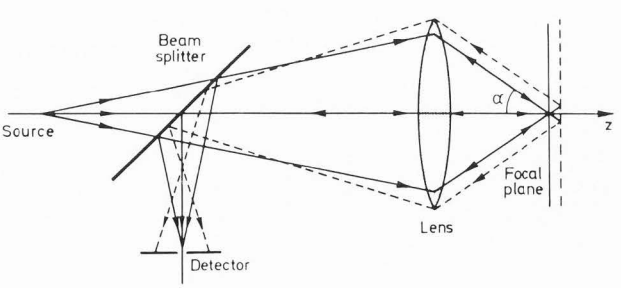

Fig.1. Schematic diagram of a confocal microscope illustrating, also, the origin of the depth discrimination or optical sectioning property.

This means, for example, that the image of a single point object is the square of the image intensity in a conventional microscope. This leads us to expect high resolution imaging with the absence of artifacts [Brakenhoff et al., 1979]. It also turns out that the image formation is purely coherent and so the image of a general object of amplitude transmittance, t(x), may be written:

$$I(x) = \left| \int c(m,u)\ T(m)\ \exp - 2\pi j m\ x\ dm \right|^2 \qquad (1)$$

where T(m) is the object spectrum or Fourier transform of t(x), m is a spatial frequency and c(m,u) is the coherent transfer function. The parameter u is a normalised axial co-ordinate which allows us to include the effects of defocus. We emphasise that we are primarily discussing a reflection mode imaging here. The case of fluorescence imaging is somewhat different in that it is essentially incoherent imaging and equation (1) does not apply.

Although the increased spatial resolution is very important perhaps the most important single property of confocal microscopes concerns their imaging of details outside the focal plane. This is shown schematically in Figure 1 where we show the effects of scanning a perfect reflector through focus. When the reflector is at the focal plane the point detector measures a large signal whereas if it is moved axially from that plane a defocused patch of light falls on the point detector and consequently a much reduced signal is measured. This is the origin of the depth discrimination or optical sectioning property. A theory using equation (1) predicts that the image of a plane reflector is given by [Wilson and Carlini, 1988]:

$$I_{plane}(u) = |c(o,u)|^2 = \left[\frac{\sin(u/2)}{u/2}\right] \qquad (2)$$

with

$$u = \frac{8\pi}{\lambda} \cdot z \cdot \sin^2 \alpha/2 \qquad (3)$$

It is fortunate that this depth discrimination effect is stronger than the depth of focus of the microscope in the sense that detail which is imaged in a confocal system tends to be in focus [Hamilton and Wilson, 1981], the rest of the information having been rejected by the pinhole.

The sectioning property together with the rejection of the out-of-focus detail lends to a tremendous number of unique derivative confocal imaging modes which depend on the three-dimensional imaging capabilities of confocal imaging. These are discussed in detail elsewhere, but include the extended focus and autofocus methods [Wilson and Hamilton, 1982] whereby adding up images from many axial positions we can obtain an image of tunable depth of focus. It is also possible to use the instrument as a non-contacting surface profilometer [Hamilton and Wilson, 1982] and, as such it has great application in the micrometrology of thick structures on semiconductor wafers. Axial scanning also permits the production of stereo pairs by a very simple computer program [Brakenhoff et al., 1986].

The depth discrimination criterion we have proposed in equation (2) does not take into account the presence of higher spatial frequencies in the object. We can confirm that these frequencies are also discriminated against by plotting $|c(m,u)|^2$ in Figure 2, where clearly we see that the transfer function falls away very rapidly for detail outside the focal plane.

The key element in all the successful implementations of those very powerful techniques is the pinhole or point detector. It is clearly not possible to have an infinitely small point detector and so some choice has to be made as to the actual size of the detector used. It is this point which we will consider in more detail in the rest of this paper.

We note that the other critical choice which has to be made concerns the method of scanning. The principal choices involve scanning the beam, scanning the objective, scanning the object, or a suitable combination of these. The actual choice will depend to a large extent on the application for which the microscope is to be used. In some cases the speed of image acquisition may be paramount whereas in others minimising the number of optical elements, for example, may be more important. We will not consider these points in any further detail in this paper, but rather concentrate on the optical properties which are common to all these approaches.

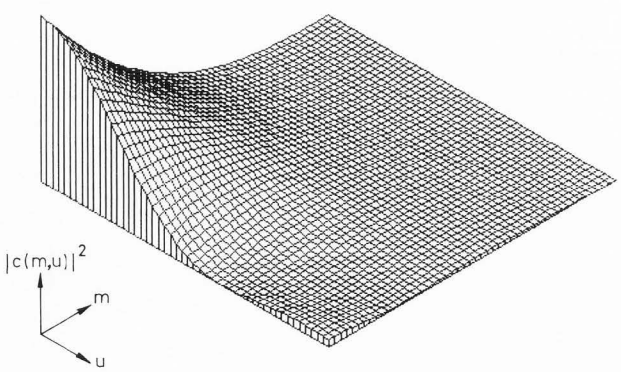

Fig.2. A representation of the transfer function $|c(m,u)|^2$.

The size of the detector

A useful design criterion is to ensure that the pinhole used is sufficiently small to give the required degree of optical sectioning. If we consider, therefore, the effect of pinhole size on the image of a perfect reflector we would expect an image given by equation (2) for an infinitely small detector and a constant signal for the case of an infinitely large detector. For finite sizes we would expect curves roughly similar in shape to equation (2) but of increasing width and without zeros as the pinhole size increased. If we take the half width of these curves as a metric of the degree of sectioning we obtain the design curve of

Figure 3. Here the full line is theoretical [Wilson and Carlini, 1987] and the dots illustrate experimental confirmation. The normalised pinhole size, v_p, is related to physical dimensions via $v_p = \frac{2\pi}{\lambda} r_p \sin^2\alpha$ where r_p is the radius of the pinhole. We note that this radius is referred to the focal plane and so the lens magnification must also be included to determine the actual pinhole size.

We note that at higher pinhole sizes the agreement is poorer than for lower values of pinhole size. This may be due to a variety of reasons. One reason is lens aberrations which will be dealt with in the next section and another is that the lens pupil function is almost certainly not ideal as was assumed in the theory. Indeed other work on the same lens has revealed that the pupil function transmittance is severely attenuated towards the edge of the pupil [Mathews, 1987].

The effect of aberrations on the sectioning

We now move on to consider the effect of the most important aberrations on the sectioning by writing the pupil function of the lens $P(\rho,\theta)$ in the form:

$$P(\rho,\theta) = \exp\tfrac{1}{2} ju\rho^2 . \exp 2\pi[A\rho^4 + B\rho^3\cos\theta + C\rho^2\cos^2\theta] \quad (4)$$

where A describes the degree of spherical aberrations, B the degree of primary coma and C the degree of primary astigmatism.

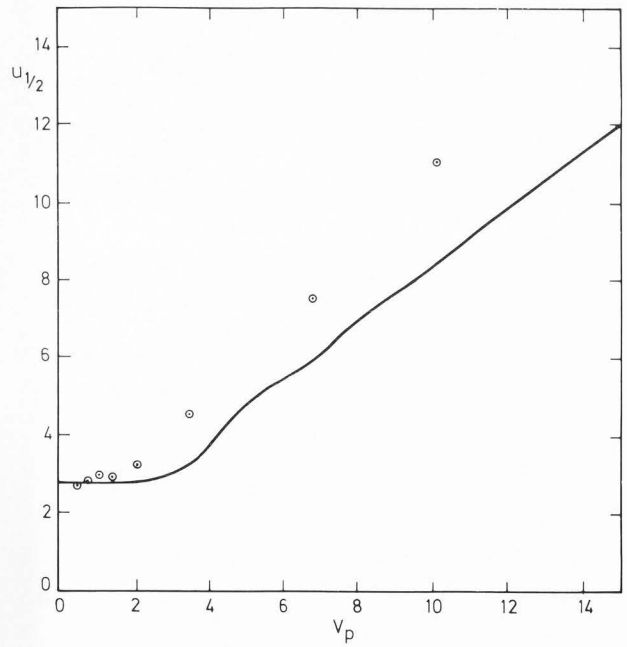

Fig.3. Halfwidth of the $I_{plane}(u)$ versus u curve for various pinhole sizes. The theoretical curve is shown as the full line and the experimental results as dots.

It is, of course, possible to discuss the role of each of these aberrations separately but it is probably more sensible at first to show the effect of all three aberrations being present. We show in Figure 4 the function $|c(m,u)|^2$ and we can see, by comparing it with Figure 2, that although a good degree of optical sectioning is still to be expected it is not as strong as in the aberration free case. If we are interested in the image of the plane reflector scanning through focus we can evaluate that via:

$$I_{plane}(u) = \int_0^{2\pi}\int_0^1 P(\rho,\theta)\, P(\rho,\pi-\theta)\, \rho\, d\rho\, d\theta \quad (5)$$

and it is clear that if all aberrations are present this will not be a symmetric function of u. We note that if a non-uniform pupil function were also to be modelled we would simply need to multiply $P(\rho,\theta)$ by the non-uniform function and under these circumstances it is very unlikely that the response would ever be symmetrical in u in the presence of any aberration.

As an example of the effect of spherical aberration on the imaging we performed an experiment similar to that used to obtain the data of Figure 3 but this time we used an objective which had a collar which could be adjusted for imaging through a certain thickness of glass. We did not use any glass, but rather used this setting to introduce a known amount of spherical aberration. Figure 5 shows the results when the aberration was corrected as accurately as possible and also where it was set equivalent to a 5 thou thickness of glass. Again it is clear that the higher the detector pinhole size the worse the effect of the aberrations. This is further emphasised in Figure 6 where we plot the half width of the $I_{plane}(u)$ versus u curves against glass thickness (which is proportional to A) for a variety of detector pinhole sizes. Again the smaller the pinhole the less deliterious is the presence of spherical aberration.

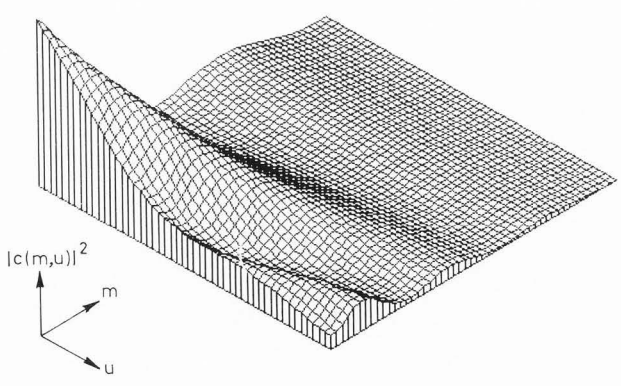

Fig.4. A representation of the transfer function $|c(m,u)|^2$ for the case of A = 0.5, B = 0.3, C = 0.3.

Conclusions

There are many unique imaging modes which arise from the use of confocal microscopy which are based on the optical sectioning or depth discrimination property which these instruments possess. The key element which turns a conventional scanning microscope into a confocal microscope is a small pinhole placed in front of the photodetector. Almost all of the three-dimensional aspects of confocal microscopy depend on this pinhole and so we have discussed some criteria by which its size may be chosen.

We have also discussed the effect of inevitable lens aberrations on the degree of sectioning we might expect. The general conclusion here is that aberrations have less effect if a sufficiently small pinhole can be used.

A further effect of the finite size of the pinhole which has been discussed elsewhere [Cox, 1986; Wilson and Carlini, 1988] is its role in the rejection of flare and scattered light. The presence of a pinhole of any size leads to an increase in the crispness of an image. If the pinhole is also chosen to be sufficiently small for true confocal operation then a dramatic increase of image quality by the rejection of stray light from various parts of the optical system is to be expected.

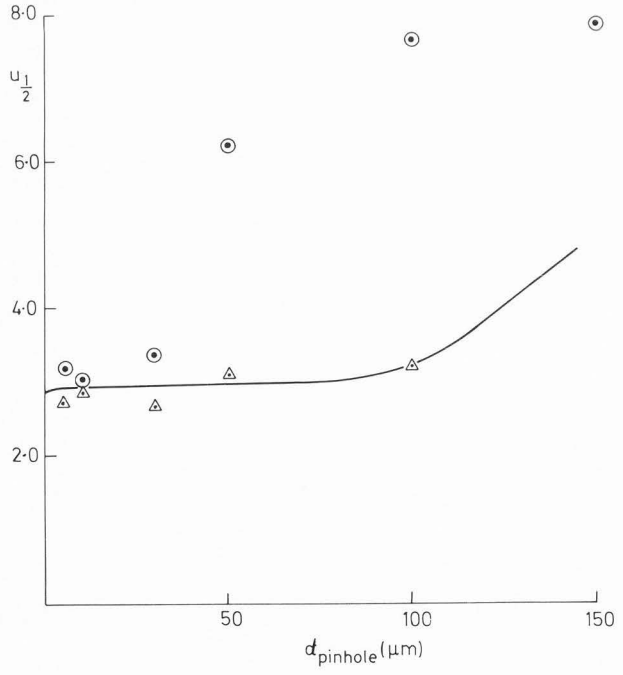

Fig.5. As in Figure 3. the points in triangles denote "zero" aberration whereas the ones in circles correspond to imaging "through" 5 thou of glass.

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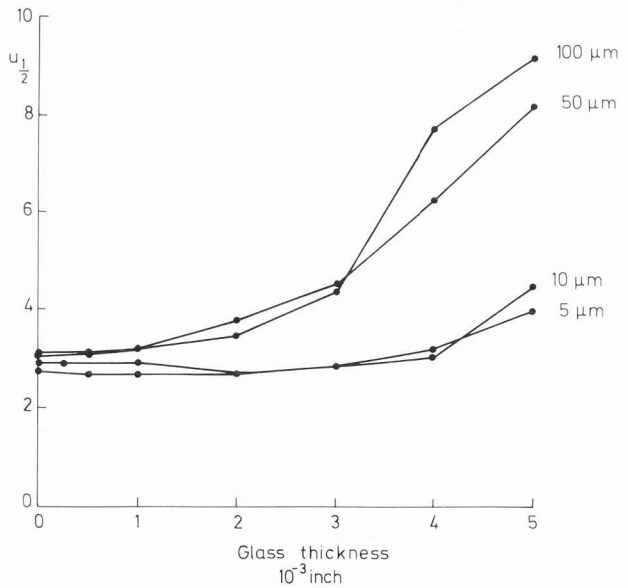

Fig.6. The variation of $u_{\frac{1}{2}}$ with glass thickness (proportional to spherical aberration) for a variety of detector pinhole sizes.

Discussion with Reviewers

J.D. Fairing: What was the focal length and N.A. of the objective used for the measurements in fig.3? If the aberrations coefficients are known will you give them?
Author: The objective had a quoted NA of 0.5 but we have found that a value of 0.44 more closely models its behaviour. I am afraid I do not know the aberration coefficients.

J.D. Fairing: What is the effect of aperture shape on the image; e.g., a square aperture covering an area equivalent to one pixel in the digitised image?
R.P. Becker: What would be the effect on imaging of using a non round "pinhole", for example a "squarish", oval or slit-shaped aperture in the detector?
Author: The use of any finite sized detector can be thought of as a compromise solution. A finite sized circular detector has the advantage of being circularly symmetric. The slit detector is the opposite extreme where we would expect different imaging properties for object features aligned parallel or perpendicular to the slit. This is most noticeable with respect to out of focus detail. Curves similar to Figure 3 can be calculated for slit detectors which show similar trends. The greatest difference is with respect to the strength of sectioning. The $I_{plane}(u)$ curve is not as narrow as that of equation (2) and does not fall off as rapidly with u. It also has no zeroes, even in theory.

The use of a square shaped detector, is used in some Nipkow disk microscopes, is better from the point of view of optical sectioning. If a comparison of equal area square and circular detectors is made the strength of sectioning is found to be essentially identical over a large range of detector sizes.

R.W. Wijnaendts van Resandt: You have shown that the correct size of the pinhole is important. Can you reach similar conclusions with respect to the actual position of the pinhole with respect to the optic axis?
Author: Similar calculations could be done to look into the effect of a misaligned finite sized pinhole but I have not done them. It is clear that the results of this paper will essentially carry over for an axially mis-aligned detector as this just gives an offset to u. We have investigated the effect of a lateral misalignment of an ideal pinhole and have found, for example that a slight misalignment can result in an enhanced image of an edge or that dark field conditions can be realised simply by positioning the detector over the first zero of the radiation pattern in the detector plane.
G.J. Brakenhoff and H.T.M. van der Voort: In off-axis confocal systems, where the beam is scanned, the effects of lens aberrations will be even more serious. Is it possible that the indicated asymmetry of the axial response changes or even changes sign over the scanfield? Does this have consequences if such off-axis systems are used for profile measurements?
Author: It is true that in a beam scanning system we would expect the axial response to change throughout the scan. However I do not expect this change to be dramatic as microscope objectives are designed to work over a finite field of view. Never the less this situation is not ideal and suggests that a non-beam scanning approach may be advantageous when ultimate performance is required.