SCANNING VOL. 19 15–19 (1997)


Received March 15, 1996
Accepted with revision June 18, 1996

# Real-Time White Light Reflection Confocal Microscopy Using a Fibre-Optic Bundle

R. JUŠKAITIS, T. WILSON, T.F. WATSON*

Department of Engineering Science, University of Oxford, Oxford; *Department of Conservative Dentistry, UMDS Guy's Hospital, London, U.K.

**Summary:** We describe a real-time white light reflection confocal microscope incorporating an optical fibre bundle and characterise the optical performance of the bundle. The use of an incoherent light source enables us, for the first time, to present speckle-free endoscopic reflected light confocal images. The system has potential application for in vivo studies.

**Key words:** confocal microscopy, endoscopy, optical fibre bundle, real-time imaging

## Introduction

The confocal microscope is a widely used instrument in many areas of biological and medical research because of its ability to image subsurface features with high resolution and contrast. The design and construction of most commercial instruments has limited their use in in vivo applications although they have been used extensively for in vitro cell culture and histologic studies. In order to be useful for clinical in vivo studies it is necessary that the confocal image be obtained in real time and that the region to be imaged be accessible with minimum patient discomfort. These requirements have essentially restricted the clinical use of confocal microscopes to the fields of ophthalmology and dentistry (Petroll *et al*. 1994, Watson 1994). In these cases it has been necessary to use special objectives with internal focussing (Petroll *et al*. 1994) so that the patient is not disturbed. However, it is not at present possible to image areas at the back of the mouth and many other inaccessible areas of the body. To achieve these goals it is necessary to develop a confocal endoscope consisting of a miniaturised objective together with a suitable fibre-optic imaging bundle.

As a first step towards the design of a clinical confocal endoscope we shall concentrate on the problems of interfacing an optical fibre imaging bundle to a real-time confocal microscope. The approach is straightforward in the sense that the proximal end of the imaging bundle is placed on the object-carrying stage of a confocal microscope. The scanning spot is then transmitted to the distal end where it is coupled into an appropriate microscope objective. The reflected or fluorescent signal is then transmitted back along the fibre bundle into the confocal microscope where the image may be viewed in real time.

Address for reprints

R. Juškaitis
Department of Engineering Science
University of Oxford
Parks Road
Oxford OX1 3PJ, U.K.

## The Optical System

Since the fibre bundle is the key element in the system, it is necessary to characterise its optical performance carefully so that the optical system may be designed correctly. We elected to use a Sumitomo IGN20/50 imaging bundle. This is a 2.1 mm diameter, 50,000 element bundle of 4 μm diameter fibres with centre-to-centre spacing of 8 μm. One requirement is that there should be minimal cross talk between adjacent fibres in the bundle. To quantify this we launched light into only one fibre of a 1 m long bundle. Figure 1 shows the light emerging from the distal end where it is clear that the light is substantially confined to a single fibre. Indeed, the ratio of the signal carried by the fibre to that carried in neighbouring fibres was measured to be −20.2 dB at most.

To characterise the individual fibres of the bundle further, we constructed the system shown in Figure 2. In this arrangement, light from an arc lamp or a laser is launched into one fibre of the bundle only. This is achieved by using a 50 μm pinhole and a lens system whose demagnification is 12.5, leading to a 4 μm diameter spot at the proximal end of the fibre which is the same size as the fibre core. At the distal end, a 6 cm focal length lens serves to collimate the light emerging from the fibre and a 32×, 0.5 NA Leitz objective is used to focus light onto the sample. Light reflected back along the fibre is directed via a beamsplitter and a 12.5× magnification lens system to a photodetector. Figure 3(a) shows the axial response obtained by measuring the detected signal, as a plane mirror specimen is scanned axially through focus for a variety of sizes of detector pinholes. The light source in this case was an Xe arc lamp together with a 488 nm interference filter. It is interesting to note that the full width half maximum of the curve corresponding to a 30 μm pinhole is 1.78 μm, whereas that predicted for an ideal confocal microscope under similar conditions is 1.61 μm. Indeed, essentially similar responses

were obtained for all pinhole sizes up to 150 μm. This may be understood by noting that for pinhole diameters < 150 μm the photodetector only detects light emerging from one fibre. We also recall that we excite only the radially symmetric modes of the fibre and that the optical sectioning behaviour of higher order modes is substantially similar to that obtained in the single mode case (Wilson 1993).

As the size of the detector pinhole is increased beyond 150 μm, the photodiode is able to detect light in neighbouring fibres. Clearly, very little of this light can be detected with a 200 μm diameter pinhole, and so we would expect almost ideal sectioning. As the pinhole size is increased, light in the neighbouring fibres begins to contribute significantly to the detected signal. It is important to note that a substantial amount of light is not coupled into the neighbouring fibres until the mirror at the distal end of the bundle is sufficiently defocussed. A simple argument, based on geometric optics, predicts that the defocus needs to be greater than about 2 μm in our case. This suggests that for defoci less than a couple of microns the optical sectioning is essentially due to the fibre and is expected to be independent of the detector pinhole size. This is borne out in Figure 3(a) where we also show the axial response measured in the absence of a pinhole altogether. The central portion exhibits optical sectioning which disappears as light couples into neighbouring fibres and is all detected by the photodetector. We note that repeating this experiment with a 633 nm wavelength filter produced substantially similar results but scaled for the longer wavelength.

It is interesting to note (Fig. 3b) that if we now repeat the experiment with a 488 nm wavelength $Ar^+$ laser as light source, we find the axial responses are essentially identical to those obtained with the arc lamp and laser-line filter. This is due to the orthogonality of the circularly symmetric modes which removes any intermodal interference.

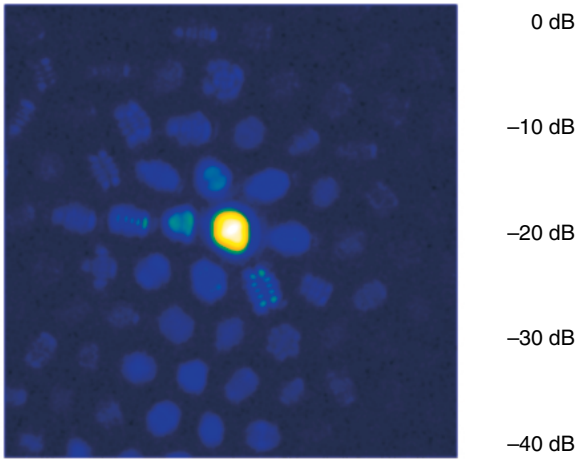



FIG. 1 Image of the distal end of the fibre bundle when light (488 nm wavelength) is launched into only one fibre of the bundle. The cross-talk between neighbouring fibres is seen to be minimal.

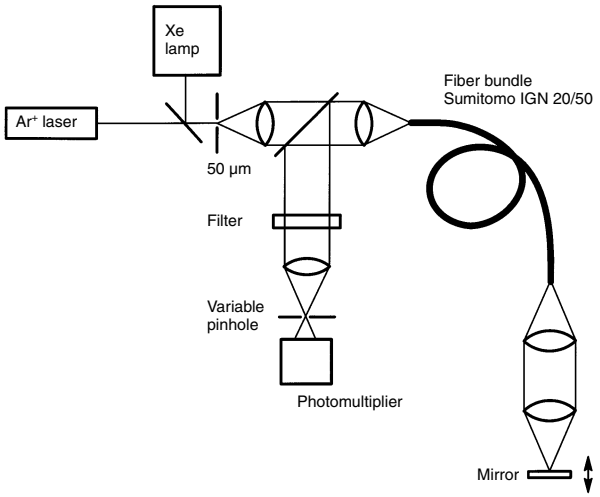



FIG. 2 Schematic diagram of the optical system used to obtain axial responses from the fibre bundle.

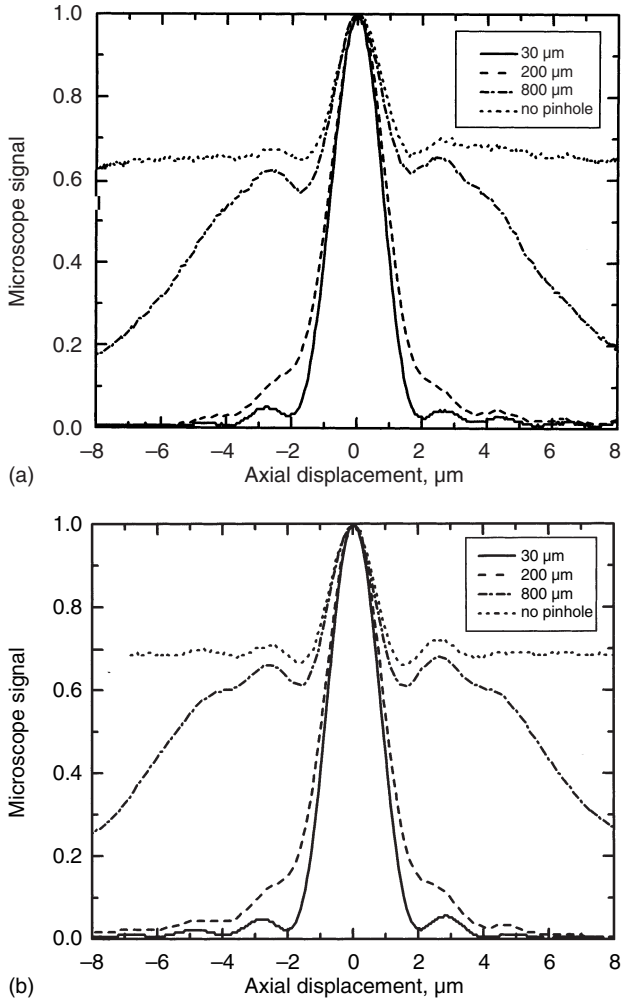



FIG. 3 (a) Axial responses from the fibre bundle for a variety of pinhole sizes. The light source was an Xe arc lamp together with a 488 nm interference filter. (b) Axial responses from the fibre bundle for a variety of pinhole sizes. The light source was a 488 nm wavelength $Ar^+$ laser.

Since it is essential for clinical applications that the confocal image be obtained in real time, the choice of host confocal microscope is effectively limited to laser-based systems using, for example, acousto-optic beam deflectors or white-light spinning disc systems. Gmitro and Azizz (1993) used a laser-based Zeiss LSM10 confocal microscope together with a 10,000 element fibre bundle to obtain fluorescence images. Our first approach, therefore, consisted of replacing the Zeiss LSM10 with a real-time, laser-based confocal microscope, Lasertec 1LM11, and the Sumitomo IGN 20/50 imaging bundle. A low-power, 6 cm focal length lens was used to couple light into the bundle. This lens was chosen so as to match the microscope's field of view to the diameter of the fibre. A collimating lens and microscope objective were used at the distal end to image the fibre bundle onto the specimen. Figure 4 shows the image of a U.S. Air Force test target taken with this system together with a 50×, 0.8 NA Olympus objective. It is immediately apparent that this reflected-light image suffers from an unacceptable degree of speckle due to the multimoded nature of the fibre bundle. This may be further understood from Figure 5(a), which is an image of the distal end of the bundle showing the light emerging from the individual fibres of the bundle. It is clear that the illumination is far from uniform and that acceptable reflected-light images will not result from this system. It is therefore necessary to consider using an incoherent arc lamp source. Figure 5(b) shows a corresponding image of the fibres at the distal end of the bundle when the $Ar^+$ laser is replaced by the Xe arc lamp and a 488 nm interference filter. The illumination is now much more uniform, and acceptable reflected-light images are more likely to result. The multimoded nature of the fibres is also clearly seen in this image.

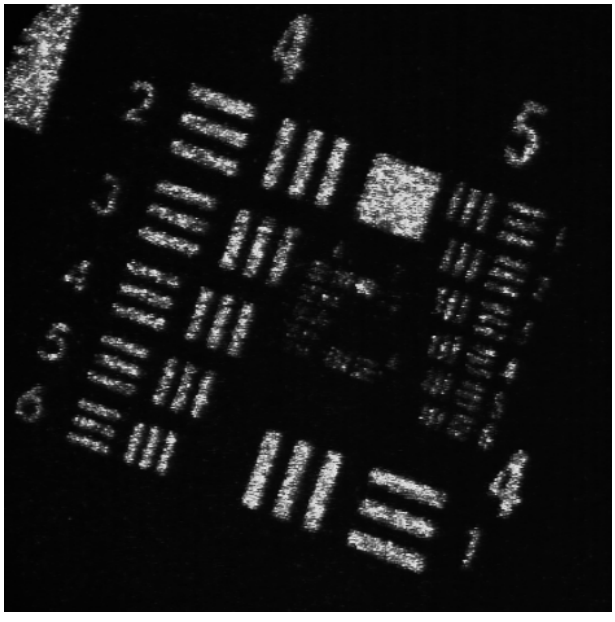
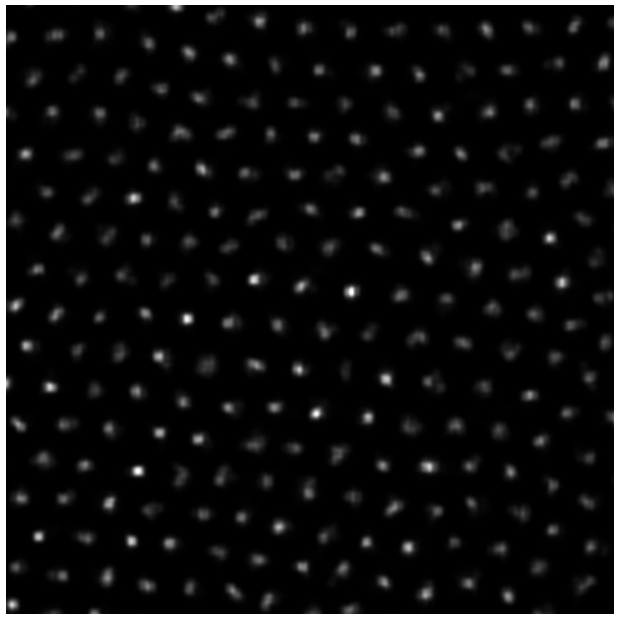
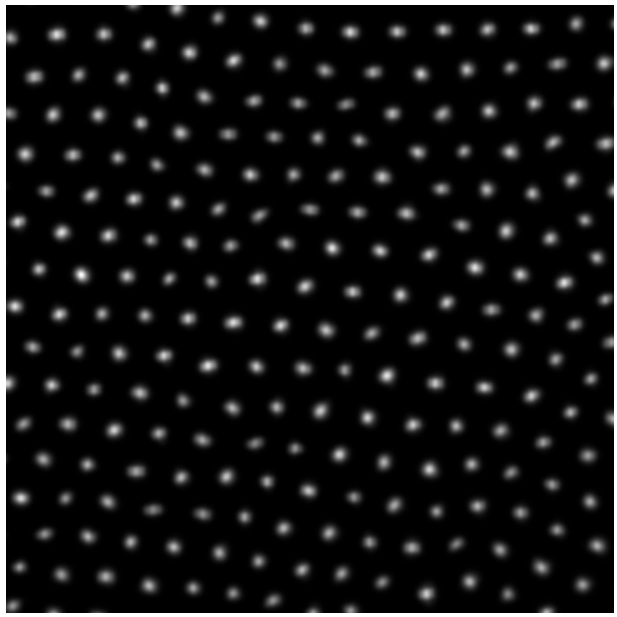


FIG. 4 The image of a U.S. Air Force test target obtained using a real-time laser-based confocal microscope. The field size is 1.2 mm.

Our final system consisted of a NORAN tandem scanning microscope, which had been configured to be "sideways looking" in the sense that the objective was mounted horizontally rather than vertically. A low-power 5×, 0.1 NA Olympus objective was used to couple light into the fibre bundle as in Figure 5. This objective lens was chosen so that the pinholes of the Nipkow disc received light from only one fibre of the bundle at any one time. The choice of optics at the distal end is determined by the tube length of the microscope objective



(a)



(b)

FIG. 5 The image of the distal end of a 1 m long fibre bundle showing the light distribution in the individual fibres of the bundle. (a) Highly uneven distribution when an $Ar^+$ laser (488 nm) is used as the light source. (b) Result of replacing the $Ar^+$ laser with an Xe arc lamp and a 488 nm interference filter.

to be used. In our case we decided to use 16 cm tube length lenses, and so it was necessary to introduce a lens such that the scanning spots emerging from the fibre appeared to originate 16 cm in front of the objective. We accomplished this with a 6 cm focal length lens. It was convenient to place this 2 cm behind the objective which then set the distance between the lens and the fibre at 4.2 cm. Angle lapping together with index matching was also used at the distal end to prevent unwanted reflections reducing image contrast. In practice, the real-time confocal images could be viewed directly by eye, or alternatively captured by a SIT camera and recorded either on video tape or using a frame grabber. The fibre bundle was 1 m in length.

The other problem to be overcome is to prevent reflections from the proximal end of the fibre passing back through the apertures in the Nipkow disc and thereby reducing image contrast. We achieve this with the system shown in Figure 6 with involves angle lapping and tilting the bundle together with index matching. To prevent reflections from the coverglass reentering the objective, it is necessary to choose the angle $\alpha > \sin^{-1}(\mathrm{NA})$, where NA represents the numerical aperture of the final microscope objective. This choice of $\alpha$ then determines the angle $\beta$ required such that the bundle front surface remains in focus, that is, $\beta = (n-1)\alpha$. It is further necessary to tilt the bundle so as to achieve on-axis illumination, that is, $\gamma = \alpha/n$. In our final design, an Olympus 5×, 0.1 NA objective was used, and so we chose $\alpha = 6^\circ$ which lead to the requirement to polish the fibre at an angle, $\delta$, of 5°.

To demonstrate the confocal optical sectioning ability of our system, we elected to begin by imaging an integrated circuit since its dimensions, both laterally and axially, were well known. Figure 7 shows images taken with white light at focal settings 3 μm apart. A 60×, 1.4NA oil immersion objective lens (Nikon) was used, and the sectioning, which is clearly visible, confirms the confocal behaviour of our combined system.

Figure 8 shows an image of an amalgam/tooth interface using a 20×, 0.8 NA oil immersion objective (Olympus). The reflective amalgam restoration can be seen at the bottom image whilst the parallel 1 μm diameter dentine tubules make up the rest of the image. These structures would not be obvi-

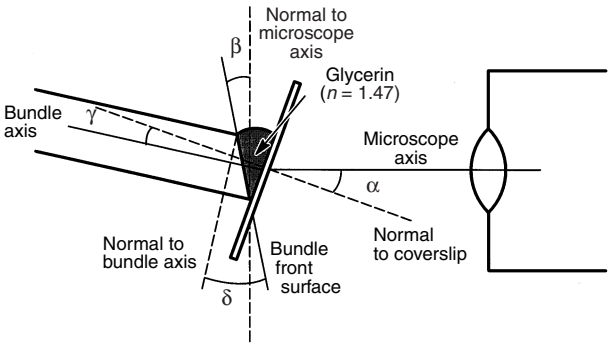



FIG. 6 Optical arrangement to prevent back reflections from the fibre bundle.

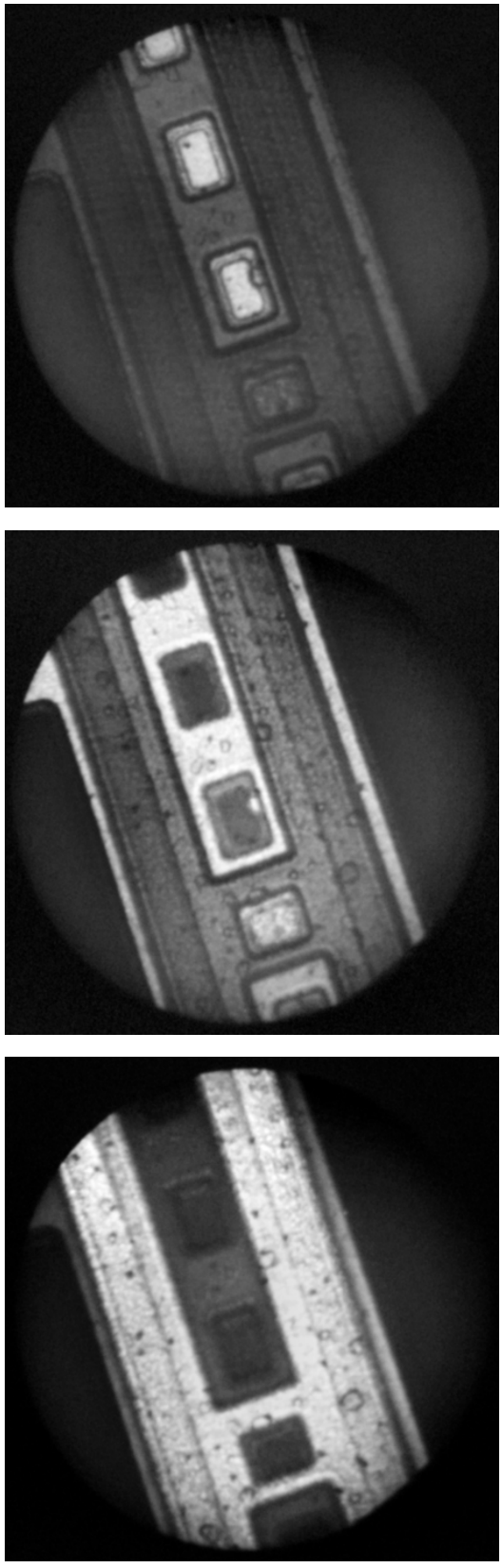


FIG. 7 A through focus series of a portion of an integrated circuit. The images were taken at focal settings 3 μm apart. The optical sectioning is clearly seen. The field diameter is 96 μm.

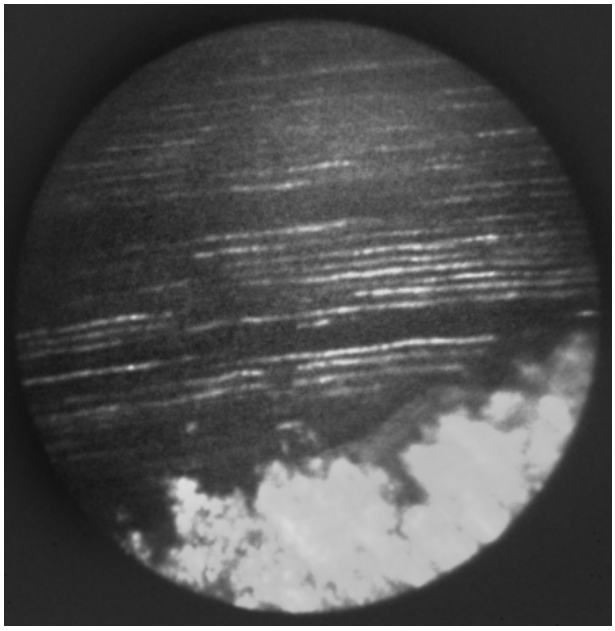


FIG. 8 Image of an amalgam/tooth interface taken with a 20×, 0.8 NA oil immersion Olympus objective. The field diameter is 280 μm.

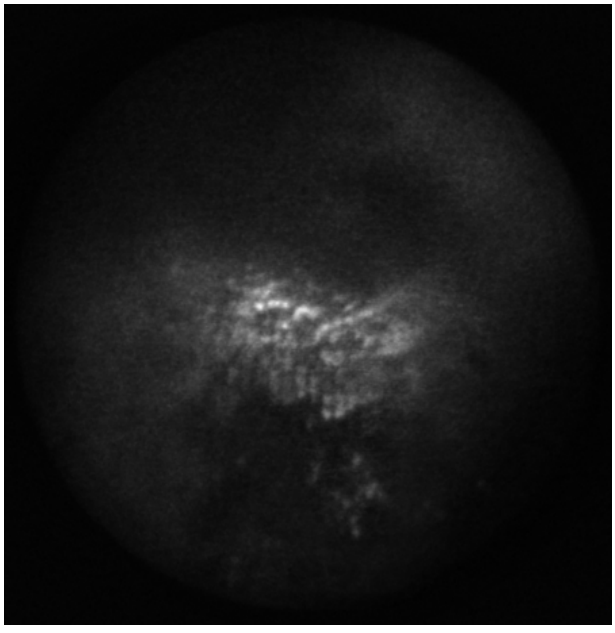


FIG. 9 Image of tooth enamel taken with a 20×, 0.8 NA oil immersion Olympus objective. The field diameter is 280 μm.

ous without a confocal imaging capability. Finally, we show in Figure 9 an image taken from the slightly curved surface of a tooth, looking into the enamel. The centre of the field shows rows of enamel prisms close to the surface of the sample, with the periphery of the image out of focus and hence appearing dark.

## Conclusions

We have described an approach aimed at producing a microscope system which will be capable of obtaining in vivo confocal images from specimens remote from the microscope. Our approach has been driven by the requirement that the image must be obtained in real time and that the system must work in both reflected light and fluorescence. The requirement for real-time imaging may be met by using either a laser-based, confocal microscope with acousto-optic or resonant galvanometer beam scanning, or a white-light tandem scanning microscope. We have chosen to obtain remote imaging by using an optical fibre bundle. If, as in our case, the individual fibres of the bundle are multimoded rather than single-moded, and reflected light images are required, then a laser-based system cannot be used since speckle will dominate the images.

Our approach, therefore, has been to use an incoherent white-light source and a tandem scanning microscope together with our fibre bundle. We have characterised the optical properties of the fibre bundle and have produced the first—to our knowledge—reflected light real-time confocal endoscopic images. A further advantage of using a white light source rather than a laser is that the wavelength of light most appropriate for the particular specimen may be chosen.

## Acknowledgment

We thank the Sir Jules Thorne Charitable Trust for support in this work.

## References

Gmitro AF, Azizz D: Confocal microscopy through a fibre-optic imaging bundle. *Opt Lett* 18, 565–567 (1993)

Petroll WM, Jester JV, Cavanagh HD: In vivo confocal imaging: General principles and applications. *Scanning* 16, 131–149 (1994)

Watson TF: Applications of high-speed imaging confocal imaging techniques in operative dentistry. *Scanning* 16, 168–173 (1994)

Wilson T: Image formation in two-mode fibre-based confocal microscopes. *J Opt Soc Am* A, 10, 1535–1543 (1993)