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Short Report

Fibre optic confocal imaging (FOCI) for subsurface microscopy of the colon in vivo

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ABSTRACT

Fibre optic confocal imaging (FOCI) is a new type of microscopy which has been recently developed (Delaney et al. 1993). In contrast to conventional light microscopy, FOCI and other confocal techniques allow clear imaging of subsurface structures within translucent objects. However, unlike conventional confocal microscopes which are bulky (because of a need for accurate alignment of large components) FOCI allows the imaging end to be miniaturised and relatively mobile. FOCI is thus particularly suited for clear subsurface imaging of structures within living animals or subjects. The aim of the present study was to assess the suitability of using FOCI for imaging of subsurface structures within the colon, both in vitro (human and rat biopsies) and in vivo (in rats). Images were obtained in fluorescence mode (excitation 488 nm, detection above 515 nm) following topical application of fluorescein. By this technique the glandular structure of the colon was imaged. FOCI is thus suitable for subsurface imaging of the colon in vivo.

INTRODUCTION

The confocal microscope was introduced by Marvin Minsky (1957, 1988). A tandem scanning reflected light confocal microscope utilising a Nipkow disc was later described by Petran et al. (1968) and modified by Xiao & Kino (1987), and the first practical single-beam laser scanning microscope for confocal epifluorescence microscopy was designed by Amos et al. (1987). The confocal microscope is now revolutionising many areas of biological and medical research (see e.g. White et al. 1987; Fine et al. 1988; review by Paddock, 1991), but until now its potential has been limited by its bulk and cost.

A conventional microscope cannot produce clear subsurface images from translucent tissues: blur and flare from out-of-focus planes obscure the image. This is the reason why tissues must be sliced into thin sections for use under conventional microscopes. Confocal microscopes eliminate the problem of blur and flare by scanning a point of light in a focal plane beneath the surface of a translucent object, only collecting light which returns back (through a pinhole) from that point (see e.g. Wilson & Sheppard, 1980). They produce an 'optical slice' through the tissue either in real time (tandem scanning instruments) or with the aid of a computer (laser scanning instruments). The major limitation of conventional commercial laser scanning confocal microscopes is that bulky optical components (which may include a laser, photomultiplier tube, beamsplitter and scanning optics) must all be accurately aligned and rigidly mounted onto a microscope. This results in an instrument which is cumbersome and easily misaligned. In addition, there is little potential for miniaturisation of such instruments.

A new type of confocal microscope has been developed which replaces the pinhole with an optical fibre. Many problems associated with optical alignment of bulky components have been eliminated, and the imaging end can now be readily miniaturised and made portable (Delaney et al. 1993). Since there is no longer a necessity for the imaging end to be accurately aligned with the bulky laser light source and detection units, this new technology (fibre optic confocal imaging, FOCI) is particularly suited to in vivo

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imaging of subsurface structures. Because of its potential for miniaturisation, FOCI may eventually be suitable for subsurface microscopy in vivo during endoscopy. The aim of the present study was therefore to assess the suitability of using FOCI for microscopic subsurface imaging of a commonly endoscoped organ, the colon, both in vivo and in vitro.

METHODS

Fibre optic confocal microscope

For this study, the commercially available HBH Fibrescan C900 fibre optic confocal microscope was used (HBH Technological Industries, Frankston Road, Dandenong, Victoria 3175, Australia). A schematic representation of this microscope is shown in Figure 1. Light from a laser source (e.g. argon ion laser) passes through a beamsplitter and is launched via an appropriate optical element (lens A, Fig. 1) into an optical fibre, which is single moded at the wavelengths utilised. Light emerging from the distal end of the fibre is focused by lens B ($\times 10$ objective lens, 0.25 numerical aperture (NA), ~ 8 mm focal length) into a beam, which is then passed through scanning optics (using galvanometer mirrors) to fill the rear entrance aperture of the objective lens C. The beam is then focused by lens C to a diffraction limited point beneath the surface of a translucent object. Light returning from that point by the same pathway is focused onto the distal end of the fibre, passes through the fibre in the reverse direction as the outgoing light, and is then detected by a photomultiplier tube after passing through the beamsplitter.

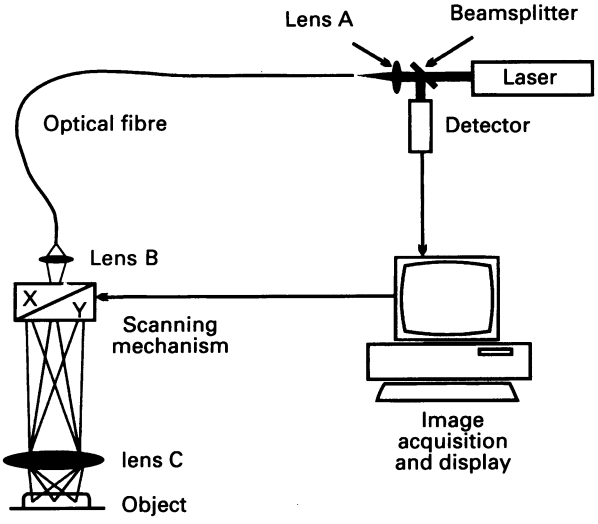

Fig. 1. Schematic representation of the HBH C900 fibre optic confocal microscope used in this study (see Methods for explanation).

Any light returning nonconfocally (i.e. from other points within the object) will not be focused on the distal end of the fibre and will not be detected to any significant extent. A 2-dimensional optical section is built up by scanning the point within the focal plane of the object in a raster pattern (using the electronically controlled scanning optics). Three-dimensional images may be reconstructed by performing 2-dimensional scans at different depths. The output from the photomultiplier tube is processed in a personal computer (IBM compatible, 386 DX 25 MHz or faster) using software developed by ROK Pty Ltd (10 Montclair Court, Templestowe, Victoria 3106, Australia) and displayed on a monitor. The lateral resolving power and depth of focus of this instrument, comparable with those of other commercially available laser scanning confocal microscopes, are dependent upon the objective lens C, and with the low power ($\times 10$, 0.25 NA) objective lens used in this study are ~ 1.5 and ~ 8 μm respectively. With a higher-power objective ($\times 60$, 1.4 NA) the lateral resolving power and depth of focus are 0.23 and 0.73 μm respectively.

In vitro experiments

Human biopsies were obtained from the sigmoid colon of 4 subjects at routine endoscopy with the understanding and consent of each subject. Rat biopsies were obtained from the sigmoid colon of 3 Monash male Wistar rats immediately after the rats had been killed by stunning and exsanguination. Biopsies were immediately placed in ice cold saline (NaCl 0.9% w/v). Within 30 min, samples were imaged in fluorescence mode, either with or without topical application of 3 drops of sodium fluorescein (100 μg/ml) to the mucosal surface, using the HBH Fibrescan C900 (2-dimensional scan time 3 s) with an argon ion laser (488 nm) with detection above 515 nm.

In vivo experiments

All experiments were performed under the aegis of the institutional committee for ethics in animal experimentation and conformed to the Australian National Health and Medical Research Council guidelines. Four Monash male Wistar rats were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.). An incision was made in the abdominal wall and in the sigmoid colon. Fibre optic confocal imaging beneath the mucosal surface of the exposed colon was performed using the HBH Fibrescan C900. A few crystals of solid sodium fluorescein dye were applied

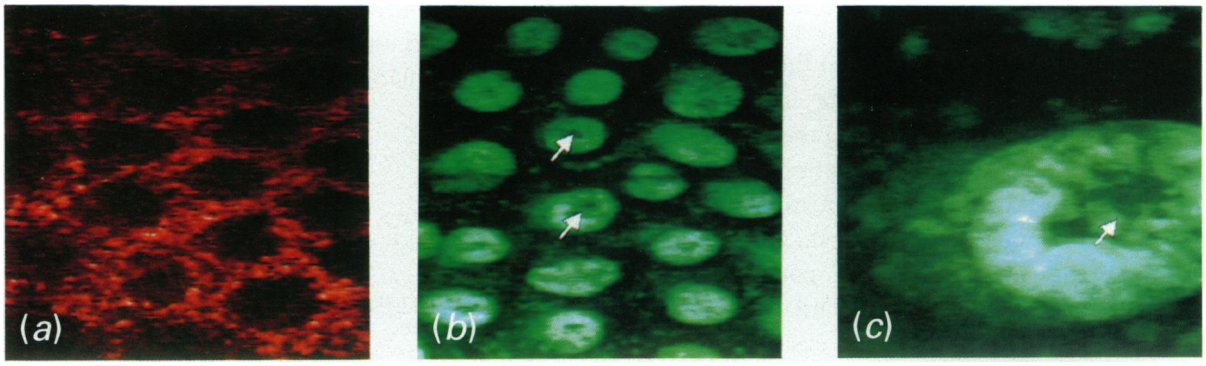

Fig. 2. Fibre optic confocal imaging of biopsies from human sigmoid colon. (*a*) Two-dimensional scan at a depth of 20 μm without the application of topical fluorescein. Illumination was from an argon ion laser (488 nm) with detection above 515 nm. The plane of the section shown is parallel with the mucosal surface of the colon (field = 300 μm) and shows autofluorescent structures within the colon. (*b*) and (*c*) Two-dimensional scans of colon biopsies 3 min after topical application of sodium fluorescein. The plane of the section shown is parallel with the mucosal surface of the colon (field size: b = 300 μm, c = 75 μm). In all cases, arrows indicate mucus-containing crypts.

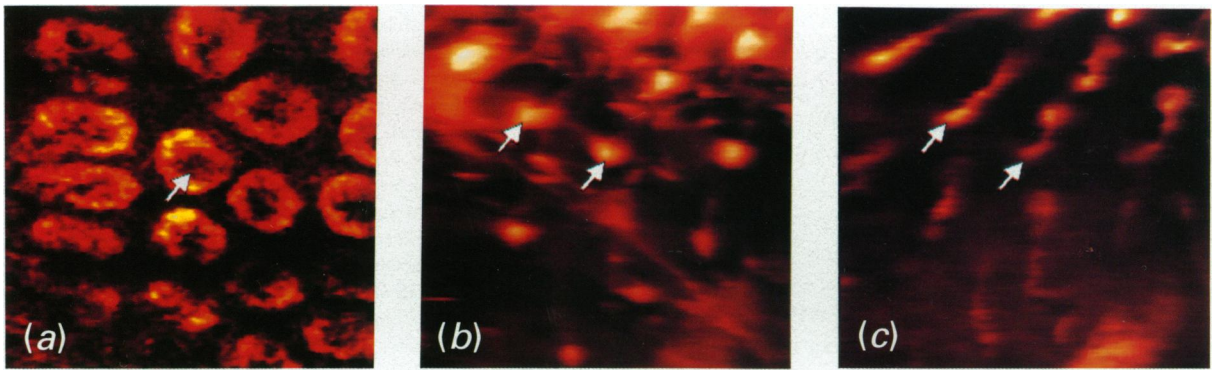

Fig. 3. (*a*) Two-dimensional scan of rat colon biopsy following topical application of fluorescein. The plane of the section is parallel with the mucosal surface, at a depth of ~ 20 μm. (*b*) Three-dimensional construct of subsurface colon structures following topical application of fluorescein to the mucosal surface of the rat colon in vivo. Six two-dimensional scans were performed (at the same site as in Fig. 2*a*) at 10 μm depth intervals, starting at 20 μm beneath the surface, to construct the computerised 3-dimensional image. (*c*) Two-dimensional scan imaged from the mucosal surface of the rat colon in vivo performed at a depth of 70 μm beneath the surface, following topical application of sodium fluorescein. In all cases, arrows indicate mucus-containing crypts, and field size = 300 μm.

to the mucosal surface, which after several minutes was washed with saline (0.9% w/v NaCl). Using a micropositioner, slight pressure was applied on the colon via a glass cover-slip beneath the objective lens to help maintain the tissue motionless prior to imaging in fluorescence mode (argon laser 488 nm, detection above 515 nm). Rat body temperature was monitored by a rectal thermometer and maintained by use of an overhead lamp.

RESULTS

Human biopsies in vitro

Figure 2*a* shows a fibre optic confocal image of a human sigmoid colon biopsy obtained in fluorescence mode without the application of topical fluorescein. This figure demonstrates that FOCI can detect autofluorescence of structures within the colon. Panels *b* and *c* of Figure 2 show images obtained using FOCI after topical application of fluorescein. The fluorescein was allowed to penetrate the tissues for approximately 3 min and, as shown in these figures, the glandular structure of the colon is clearly outlined.

Rat colon imaging in vitro and in vivo

Figure 3*a* shows a fibre optic confocal image of a rat colon biopsy following topical application of fluorescein. Panels *b* and *c* show images obtained by FOCI after topical application of fluorescein to the rat colon in vivo. These images show the glandular structure. The pattern of penetration of fluorescein in the rat in vivo appears somewhat different to its penetration of human and rat biopsies in vitro. This may reflect the in vivo functioning of colonic glands, since continued mucus production in vivo may affect penetration of the fluorescent dye into the tissue.

DISCUSSION

The results presented above show for the first time the feasibility of producing subsurface images of the colon both in vitro and in vivo using the novel type of microscopy, FOCI. Clear in vivo subsurface imaging of microscopic structures is not possible using ordinary light microscopy because of blur and flare from out-of-focus planes. Conventional confocal microscopes eliminate such blur and flare (White et al. 1987). However, they are bulky and easily misaligned, making them difficult or inconvenient to use for certain in vivo applications. Indeed, the conventional laser scanning confocal microscope was designed primarily for epifluorescent microscopy of fixed specimens (Amos et al. 1987; Paddock, 1991). The FOCI microscope offers advantages over such instruments since it allows the imaging end to be freely mobile, transportable between objective elements and miniaturised, e.g. for endoscopy. FOCI is thus particularly suitable for microscopy of subsurface structures in vivo, as demonstrated by the results of the present study.

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