Confocal Endoscopy via Structured Illumination

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Summary: We describe a simple modification to a rigid endoscope so as to provide both high-quality conventional and confocal images of reasonably accessible regions of the body. This versatile system uses a structured illumination approach together with a conventional incoherent illumination source. Images taken in fluorescence are presented using this combined conventional and confocal endoscope.

Key words: confocal microscopy, endoscopy, rigid endoscopes, real-time imaging, in vivo imaging

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Introduction

The use of endoscopes is becoming important in an increasingly wide range of medical and surgical treatments. There are broadly two classes of purely optical endoscope in clinical use. The first consists of a long flexible fibre-optic coupling between the remote site to be investigated and the user (Cotton and Williams 1996). These give adequate diagnostic information, although the image quality may be compromised by the number of elements within the bundle. An alternative class of endoscope uses a thin rigid tube enclosing an appropriate lens system (Hopkins 1975). These instruments are optically superior to their fibre bundle counterparts, although their use is confined to regions of the body that afford straight line access to the region of interest. The image contrast in these instruments originates from both the surface and subsurface regions of the translucent tissue under examination. To discriminate between these structural features in a controllable way, it is advantageous to be able to introduce confocal imaging. Fluorescent labelling may also allow greater differentiation between cell layers capable of absorbing the dye solution.

The host confocal optical system must be able to capture images in real time, and a number of attempts have been made to combine confocal microscopes with flexible endoscopes (e.g., Gmitri and Aziz 1993, Juškaitis et al. 1997). However, to our knowledge, relatively little attention has been paid to rigid endoscopy. Since the clinician is used to working with a conventional endoscope, it is desirable that the addition of confocal imaging capability is not made at the expense of conventional viewing. For this reason we have elected to provide the confocal imaging capability by using a structured illumination approach, since this implementation provides both the conventional and confocal images simultaneously (Neil et al. 1997, 2000).

Materials and Methods

The basis of the structured illumination approach to confocal microscopy lies in the observation that, in a conventional incoherent imaging system, all spatial frequencies apart from the zero spatial frequency attenuate with defocus (Neil et al. 1997). This suggests that if we modify the illumination path of an endoscope so as to project a spatially modulated pattern of light onto the tissue, then we should be able to extract the optically sectioned confocal image by appropriate analysis of the spatially modulated part of the fluorescence image.

In practice we introduce a one-dimensional grid, with an equal mark-to-space ratio (Optem, Fairport, N.Y., USA) into the illumination arm of the endoscope (Fig. 1) so as to cause a one-dimensional spatially varying fringe pattern to be projected onto the tissue. Images are taken at three spatial positions corresponding to a relative movement of one third of the grid period of the grid between images are then processed to extract both the confocal and conventional image. The details of the processing have been described elsewhere (Neil et al. 1997, 2000).

The rigid endoscope we used was a Hopkins type contact telescope (Karl Storz, Tuttlingen, Germany) which contained an internal focussing mechanism and a 30° forward angle lens for imaging structures lateral to the axis of the endoscope. A 10-bit digital CCD camera (DVC-1300, DVC, Austin, Tex., USA) was attached to the endoscope for final image capture. Further, direct viewing of the images was also available.
Results

To demonstrate the optical sectioning ability of our endoscope system, we constructed a step test specimen consisting of two microscope cover slips (#3) sitting on a microscope slide. The whole structure was covered with a layer of fluorescent dye. The height difference between the fluorescent layers was 600 µm. Figure 2 shows the conventional and confocal images obtained when focussing on the top and bottom layer. A standard Xenon microscope incoherent illumination source was used together with fluorescence filter cube (U-MSWB, Olympus America, Inc., Melville, N.Y., USA) which comprised a 420–480 nm excitation filter, a 500 nm dichroic beamsplitter, and a 515 nm long-pass detection filter. As might be expected, the conventional image remains essentially unchanged at both focal settings, whereas the confocal images demonstrates optical sectioning very clearly. It is impossible to predict theoretically the optical sectioning strength, since the numerical aperture of the endoscope is regarded by the manufacturer as proprietary information; however, our measurements would suggest a value of 0.06, which corresponds to the optical sectioning strength observed.

Figure 3 shows a confocal and conventional pair of fluorescence images of the keratinised epithelium of the palmar aspect of a finger tip. Experiments were carried out with range of fluorescent dyes. Acceptable results were obtained with fluorescence. However, to our surprise, the use of a common office yellow marker pen to apply dye to the region of interest gave by far the strongest fluorescence signal levels and hence produced the best images. In all cases glycerol was used as a coupling medium. The alternating light and dark banding that can be seen across the field (Fig. 3) reflects the gross ridge and trough convolutions of the basement membrane level associated with the ridge and trough pattern of the overlying finger print. Sweat ducts may also be clearly discerned in the images. The confocal image shows greater contrast and resolution than its conventional counterpart. Figure 4 shows a similar confocal and conventional pair of fluorescence images taken near the surface of the wrist. Again, a considerably crisper and sharper image is obtained in the confocal case.

It is important to realize that the commercial clinical endoscope used here is designed to provide widefield images.
and therefore is not ideal for use in a confocal application where higher numerical aperture—and hence stronger optical sectioning and higher resolution—is desired. Although reflected or scattered light imaging is perfectly possible in principle using the structured illumination approach, it is not possible to achieve this in practice using this particular endoscope due to the wide-angle nature of the imaging lens. It would, however, be perfectly possible to overcome this drawback by designing a suitable telecentric lens to replace the wide-angle optics in the existing endoscopes. This approach is facilitated by the fact that the internal optics of the Hopkins system make use of much higher numerical apertures than those afforded by the distal end optics of the current clinical device.

**Conclusions**

We have demonstrated a simple modification that permits both confocal and conventional images to be obtained
from a clinical endoscope. Since the system must operate in real time to be clinically useful, we have elected to implement the confocal imaging via a structured illumination approach since this has the added flexibility of permitting standard incoherent light sources rather than a laser to be used. A further advantage of the structured illumination approach is that the familiar conventional image is also produced simultaneously. A number of preliminary images, which confirm the viability of this approach have been produced.

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References


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