Nonlinear optical endoscopy allows 3D deep tissue imaging

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Double-clad photonic crystal fibers and microelectromechanical mirrors significantly improve the sensitivity and miniaturization of nonlinear optical endoscopes.

During the last two decades, nonlinear optical microscopy (NLOM) has emerged as a rapidly-growing new optical engineering field. The technique is primarily based on multiphoton-excited fluorescence combined with second- or third-harmonic generation and can provide images inside a thick specimen with sub-micrometer resolution. The main advantage of NLOM is the ability to maintain resolution and contrast within scattering tissues. A drawback, however, for in vivo applications has been the bulky optical microscopy instrumentation that prevents the imaging of internal organs in intact and moving animals. Hence, the development of miniature nonlinear optical microscopes and endoscopes that allow imaging under conditions unfeasible for a conventional microscope has attracted a high level of interest. The design of such devices also has the potential of expanding optical imaging modalities to cancer monitoring and minimally invasive surgery.

Key challenges, however, must be met before these devices can be fully exploited. One of the most important is the high sensitivity required for biological and clinical applications. Laser scanning mechanisms must also be efficiently miniaturized, and flexible and compact imaging probes must be designed. Efforts addressing these technical issues have recently been reported. For example, miniaturized scanning mechanisms such as piezoelectric actuators and fiber-optic devices such as single-mode fibers and hollow-core photonic-crystal fibers (PCFs) have been proposed. However, currently available miniature NLOM devices are not yet suitable for endoscopic operation. This is due to a lack of flexibility in the overall system design or to fiber-optic devices that are inefficient for both excitation beam delivery and signal collection.

We have recently used double-clad PCFs and microelectromechanical systems (MEMS) mirrors to fabricate a prototype nonlinear optical endoscope that represents a significant breakthrough in high sensitivity in vivo imaging.

We first used a custom-designed double-clad PCF with an inner cladding of 165µm and a numerical aperture of 0.6. Unlike other fibers, it offers the single-mode delivery of near infrared light in the central core combined with the efficient propagation of visible light through the inner cladding in a single fiber (see Figure 1). This allowed us to overcome the characteristic low signal levels of conventional fiber-optic microscopy systems. A coupling efficiency of up to 90% in the 410 to 800nm-wavelength range is achievable. When compared to conventional fibers, use of the double-clad PCF enhanced the signal level of the endoscopy system by two orders of magnitude.

To steer the beam out of the PCF, we used a MEMS mirror fabricated by sequential material etching and deposition processes. Further, the bulk imaging objective was replaced by a gradient-
Compared to a NLOM device using a single-mode fiber coupler and a GRIN lens, the signal level was increased by a factor of approximately 160 as a result of the large collection area and the high numerical aperture of the double-clad PCF.

In our most recent design, the laser beam is steered from the fiber by a 2D MEMS mirror and focused on a sample through a GRIN lens to form an image. The MEMS mirror is based on electrothermal actuation and can perform large bidirectional 2D optical scans. Backscattering nonlinear optical signals are propagated through the same fiber and collected by the detector. This arrangement allows the endoscope head to have a diameter of approximately 3mm after the integration of the MEMS mirror and the GRIN lens. Our new nonlinear optical endoscope system was evaluated during a series of comprehensive 3D tissue imaging experiments. Results showed that it could achieve a penetration depth of approximately 100µm and an axial resolution of some 10µm.

In conclusion, a nonlinear optical microscope system based on double-clad PCFs, MEMS mirrors, and GRIN lenses was successfully used for the 3D imaging of deep tissues. Future work will focus on the integration of a double-clad PCF coupler to further increase the flexibility of the light path and on the engineering of double-clad PCFs to compensate for pulse delivery dispersion effects. The further development of a tiny endoscope head will also enable in vivo imaging for optical biopsy and early cancer detection applications.

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References