Dual modality endomicroscope with optical zoom capability

Dimitre G. Ouzounov,1,* David R. Rivera,1 Wendy O. Williams,2 John A. Stupinski,3 Teresa L. Southard,3,4 Kelly H. Hume,3,5 Julie Bentley,6 Robert S. Weiss,3 Watt W. Webb,1 and Chris Xu1

1School of Applied and Engineering Physics, Cornell University, Ithaca, NY, 14853, USA
2College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA
3Department of Biomedical Sciences, Cornell University, Ithaca, NY, 14853, USA
4Department of Pathology, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA
5Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853 USA
6The Institute of Optics, University of Rochester, Rochester, NY USA
*Corresponding author: dgo4@cornell.edu

Abstract: We present a miniature endomicroscope that combines large field-of-view (FOV) (1.15 mm) reflectance imaging with high-resolution (~0.5 μm) multiphoton intrinsic fluorescence imaging. We acquired in vivo and ex vivo images of unstained normal and tumor-laden tissues by using the large-FOV mode to navigate to the site of interest and then switching to the high-resolution modality to resolve cellular details.

©2013 Optical Society of America

OCIS codes: (170.2150) Endoscopic imaging; (110.2350) Fiber optics imaging; (180.4315) Nonlinear microscopy.

References and links

1. W. Denk, J. H. Strickler, and W. W. Webb, “Two-photon laser scanning fluorescence microscopy,” Science 248(4951), 73–76 (1990).
2. W. R. Zipfel, R. M. Williams, and W. W. Webb, “Nonlinear magic: multiphoton microscopy in the biosciences,” Nat. Biotechnol. 21(11), 1369–1377 (2003).
3. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. Fujimoto, “Optical coherence tomography,” Science 254(5035), 1178–1181 (1991).
4. M. Minsky, “Memoir on inventing the confocal scanning microscope,” Scanning 10(4), 128–138 (1988).
5. R. Kiesslich, M. Goetz, M. Vieth, P. R. Galle, and M. F. Neurath, “Confocal laser endomicroscopy,” Gastrointest. Endosc. Clin. N. Am. 15(4), 715–731 (2005).
6. J. F. Xi, Y. Chen, Y. Zhang, K. Murari, M. J. Li, and X. Li, “Integrated multimodal endomicroscopy platform for simultaneous en face optical coherence and two-photon fluorescence imaging,” Opt. Lett. 37(3), 362–364 (2012).
7. E. J. Seibel and Q. Y. Smithwick, “Unique features of optical scanning, single fiber endoscopy,” Lasers Surg. Med. 30(3), 177–183 (2002).
8. B. A. Flusberg, J. C. Jung, E. D. Cocke, E. P. Anderson, and M. J. Schnitzer, “In vivo brain imaging using a portable 3.9 gram two-photon fluorescence microendoscope,” Opt. Lett. 30(17), 2272–2274 (2005).
9. H. C. Bao, J. Allen, R. Pattie, R. Vunc, and M. Gu, “Fast handheld two-photon fluorescence microendoscope with a 475 μm x 475 μm field of view for in vivo imaging,” Opt. Lett. 33(12), 1333–1335 (2008).
10. Y. C. Wu, J. F. Xi, M. J. Cobb, and X. D. Li, “Scanning fiber-optic nonlinear endomicroscopy with miniature aspherical compound lens and multimode fiber collector,” Opt. Lett. 34(7), 953–955 (2009).
11. D. R. Rivera, C. M. Brown, D. G. Ouzounov, I. Pavlova, D. Kobat, W. W. Webb, and C. Xu, “Compact and flexible raster scanning multiphoton endoscope capable of imaging unstained tissue,” Proc. Natl. Acad. Sci. U.S.A. 108(43), 17598–17603 (2011).
12. P. Kim, E. Chung, H. Yamashita, K. E. Hung, A. Mizoguchi, R. Kucherlapati, D. Fukumura, R. K. Jain, and S. H. Yun, “In vivo wide-area cellular imaging by side-view endomicroscopy,” Nat. Methods 7(4), 303–305 (2010).
13. M. T. Myaing, D. J. MacDonald, and X. D. Li, “Fiber-optic scanning two-photon fluorescence endoscope,” Opt. Lett. 31(18), 1076–1078 (2006).
14. L. Fu, A. Jain, C. Cranfield, H. K. Xie, and M. Gu, “Three-dimensional nonlinear optical endoscopy,” J. Biomed. Opt. 12(4), 040501 (2007).
15. D. Bird and M. Gu, “Two-photon fluorescence endoscopy with a micro-optic scanning head,” Opt. Lett. 28(17), 1552–1554 (2003).
16. G. J. Liu, T. Xie, I. V. Tomov, J. Su, L. Yu, J. Zhang, B. J. Tromberg, and Z. Chen, “Rotational multiphoton endoscopy with a 1 μm fiber laser system,” Opt. Lett. 34(15), 2249–2251 (2009).
17. C. I. Engelsbeck, R. S. Johnston, E. J. Seibel, and F. Helmchen, “Ultra-compact fiber-optic two-photon microscope for functional fluorescence imaging in vivo,” Opt. Express 16(8), 5556–5564 (2008).
18. R. P. Barretto, B. Messerschmidt, and M. J. Schnitzer, “In vivo fluorescence imaging with high-resolution microlenses,” Nat. Methods 6(7), 511–512 (2009).
19. C. Liang, K. B. Sung, R. R. Richards-Kortum, and M. R. Descour, “Design of a high-numerical-aperture miniature microscope objective for an endoscopic fiber confocal reflectance microscope,” Appl. Opt. 41(22), 4603–4610 (2002).
20. S. M. Landau, C. Liang, R. T. Kester, T. S. Tkaczyk, and M. R. Descour, “Design and evaluation of an ultra-slim objective for in-vivo deep optical biopsy,” Opt. Express 18(5), 4758–4775 (2010).
21. K. Carlson, M. Chidley, K. B. Sung, M. Descour, A. Gillenwater, M. Follen, and R. Richards-Kortum, “In vivo fiber-optic confocal reflectance microscope with an injection-molded plastic miniature objective lens,” Appl. Opt. 44(10), 1792–1797 (2005).
22. R. T. Kester, T. S. Tkaczyk, M. R. Descour, T. Christenson, and R. Richards-Kortum, “High numerical aperture microendoscope objective for a fiber confocal reflectance microscope,” Opt. Express 15(5), 2409–2420 (2007).
23. D. G. Ozoumov, D. R. Rivera, W. W. Webb, J. Bentley, and C. Xu, “Miniature varifocal objective lens for endomicroscopy,” Opt. Lett. (to be published).
24. M. Gu, J. R. Sheppard, and H. Zhou, “Optimization of axial resolution in confocal imaging using annular pupils,” Optik (Stuttg.) 93, 87–90 (1993).
25. J. R. Sheppard, “The use of lenses with annular aperture in scanning optical microscopy,” Optik (Stuttg.) 48, 329–334 (1977).
26. S. S. Mukherjee, J. S. Wysock, C. K. Ng, M. Akhtar, S. Fenn, M. Lee, M. Rubin, F. Maxfield, W. Webb, and S. Scherr, “Human bladder cancer diagnosis using Multiphoton microscopy,” Proc. SPIE 7161, 9639 (2009).
27. I. Pavlova, K. R. Hume, S. A. Yazinski, J. Flinders, T. L. Southard, R. S. Weiss, and W. W. Webb, “Multiphoton microscopy and microspectroscopy for diagnostics of inflammatory and neoplastic lung,” J. Biomed. Opt. 17(3), 036014 (2012).
28. W. R. Zipfel, R. M. Williams, R. Christie, A. Y. Nikitin, B. T. Hyman, and W. W. Webb, “Live tissue intrinsic emission microscopy using multiphoton-excited native fluorescence and second harmonic generation,” Proc. Natl. Acad. Sci. U.S.A. 100(12), 7075–7080 (2003).
29. E. L. Jackson, N. Willis, K. Mercer, R. T. Bronson, D. Crowley, R. Montoya, T. Jacks, and D. A. Tuveson, “Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras,” Genes Dev. 15(24), 3243–3248 (2001).
30. R. Le Harzic, M. Weinigel, I. Riemann, K. König, and B. Messerschmidt, “Nonlinear optical endoscope based on a compact two axes piezo scanner and a miniature objective lens,” Opt. Express 16(25), 20588–20596 (2008).
31. F. Helmchen and W. Denk, “Deep tissue two-photon microscopy,” Nat. Methods 2(12), 932–940 (2005).
32. E. Beaurepaire and J. Mertz, “Epifluorescence collection in two-photon microscopy,” Appl. Opt. 41(25), 5376–5382 (2002).
33. R. Kiesslich and M. F. Neurath, “Endomicroscopy is born--do we still need the pathologist?” Gastrointest. Endosc. 66(1), 150–153 (2007).

1. Introduction

The standard clinical practice to assess tissue health requires the extraction of tissue samples (i.e., tissue biopsies), which are then processed into histological sections and examined for diseases using a conventional light microscope. However, tissue biopsies are often associated with patient discomfort, high costs, and delayed diagnostics. In addition, the lack of precise guidance often leads to inaccuracies in the selection of tissue regions for biopsy. Therefore, the development of an optical endoscope that provides real time, accurate tissue diagnostics in the clinic is of great importance.

Wide-field clinical endoscopes are extensively used to guide tissues biopsies and although they offer large FOV, the spatial resolution allows only for gross inspection of tissue morphology. High spatial resolution imaging modalities such as multiphoton microscopy (MPM) [1, 2], optical coherence tomography (OCT) [3], and confocal microscopy [4] can achieve high spatial resolution but when implemented into compact clinical endoscopes [5–22], these modalities provide FOVs typically only hundreds of microns, which significantly reduces their usefulness in clinical translation. To be effective as a biopsy tool, clinical endoscopes should provide large FOV to investigate a large tissue area and cellular resolution to deliver diagnostic quality imaging. Because a miniature endoscopic lens cannot achieve these two requirements simultaneously, optical zoom capability is an indispensable functionality for a clinical endoscope. For conventional bench top light microscopes, optical
This mechanical approach is very difficult to miniaturize, and for practical consideration both large FOV modality and high-spatial resolution modality should be implemented within the same endoscope without mechanical adjustments of the distal parts. An optical endoscope that provides high-spatial resolution imaging with optical zoom capability has never before been demonstrated.

Here, we present a dual modality endomicroscope that provides high-resolution imaging through multiphoton modality and large FOV imaging through single photon reflection/scattering modality.

2. Endomicroscope design and characterization

2.1 Endomicroscope design

The essential element of the endomicroscope is a 3 mm outside diameter (OD), catadioptric zoom lens [23] based on the principle of wavelength division multiplexing. The optical zoom operation is achieved by changing the wavelength of the excitation light without any mechanical adjustment at the endomicroscope distal end.

We integrated this zoom lens with a previously demonstrated [11] miniaturized resonant/non-resonant fiber raster scanner into a fully functional endomicroscope probe with 5 mm OD and 5 cm rigid length (Fig. 1(a)). The scanner incorporates two scanning optical fibers that are bonded alongside each other: a hollow-core photonic band-gap fiber (HC-PBGF, HC-800-2, NKT Photonics) with a transmission window at 800 nm for high-resolution multiphoton imaging, and a standard single mode fiber (SSMF) at 400 nm for large FOV, one-photon reflectance imaging. The fibers are bonded with instant adhesive (Loctite 495) and the distal end faces of the two fibers lie in the same plane. The schematic of the endomicroscope is shown in Fig. 1(b).

![Fig. 1. Dual modality endomicroscope. (a) Photograph of the endomicroscope with optical zoom. (b) Schematic illustration of the optical and mechanical configurations of the endomicroscope.](image-url)
2.2 Endomicroscope characterization

The experimental setup used for instrument characterization and for tissue imaging is shown in Fig. 2. The excitation source for high-resolution mode imaging is a mode-locked Ti:Sapphire laser (Tsunami, Spectra Physics) operating at 800 nm. To compensate for the anomalous dispersion of the hollow core photonic band-gap fiber (HC-PBGF), the femtosecond pulses are pre-chirped by passing through a piece of SF11 glass and then coupled into the HC-PBGF fiber (~1 m long, Fig. 1(b)) of the endomicroscope. We use a fiber-coupled continuous wave (CW) semiconductor laser operating at 406 nm (LP406-SF20, Thorlabs) for low-magnification, large FOV imaging. The CW laser is coupled into a 1 m long SSMF which is spliced to the SSMF of the endomicroscope (Fig. 1(b)). The scanner was operated at frame rate of 4.1 frames/s (512x512 pixels/frame). The two-photon fluorescence and one-photon reflected/scattered light are epi-collected using 10 large core (0.5 mm OD) plastic optical fibers [23], and detected by an ultra bi-alkali (R7600U-200, Hamamatsu) PMT, which has an active area of ~400 mm$^2$. Short pass filters (FF720-SDi01-25x36, Semrock) were used in front of the PMT. The PMT output current was amplified and converted to voltage (C7319, Hamamatsu), and then digitalized at 16 bits at 10 MHz by a data acquisition card (NI PCI-6115, National Instruments). Imaging acquisition and scanner control is performed with the software ScanImage.

We characterized the performance of the device by imaging a US Air Force (USAF) test target in transmission. The lateral resolution (FWHM of PSF, measured as in [23]) of the high-magnification mode is ~0.75 μm (Fig. 3(a)), which corresponds to a two-photon resolution (FWHM) of ~0.5 μm. The side lobes of the PSF are caused by the obstruction at the center of the back aperture, which is a well-known characteristic of a reflective objective lens. Because of the nonlinear nature of the signal generating process, these side lobes will be significantly suppressed in two-photon imaging. The FOV of the high-magnification mode is ~160 μm (Fig. 3(b)). The one-photon lateral resolution of the low-magnification mode was measured to be ~4.5 μm. The low-magnification imaging mode achieved a large FOV of 1.15 mm (Fig. 3(c)). The output beam of the miniature zoom lens operating in high-magnification mode has an annular profile [23], which reduces the axial resolution [24, 25]. We characterized the two-photon axial resolution of the high-magnification mode by stepping a
500-nm Rhodamine B thin film through its focus. The measured FWHM of the thin film response is ~10.5 μm (Fig. 3(d)), which is sufficient for resolving cellular layers in biological tissues. The resolution of the packaged endomicroscope is similar to that of the stand-alone catadioptric zoom lens [23], indicating that packaging of the miniature zoom lens into a fully functional zoom endomicroscope did not adversely affect its optical performance.

![Image of dual modality endomicroscope characterization](insert)

**Fig. 3. Dual modality endomicroscope characterization.** (a) Calculated (red solid line) and measured (blue dashed line) lateral point spread function for the high-resolution imaging mode. Inset: Group 9 of USAF high-resolution target imaged in transmission using the high-resolution imaging mode at 800 nm. (b) USAF resolution target imaged in transmission using the high-magnification mode (\(\lambda = 800\) nm). (c) USAF resolution target imaged in transmission using the low-magnification mode (\(\lambda = 406\) nm). (d) Axial scan of a thin Rhodamine B film showing the two-photon axial resolution (FWHM) of 10.5 micron.

3. **Ex vivo and in vivo imaging of unstained rodent tissues**

3.1. **Ex vivo imaging**

To demonstrate and test the capability of the dual modality, dual optical zoom operation, we acquired *ex vivo* images from an unstained tumor-laden mouse lung lobe (~3 mm by ~5 mm), and identified normal and abnormal tissue regions within the lobe using both imaging modalities. As indicated in Fig. 4(a), images were obtained from three different areas within the tumor-laden lobe. Figures 4(b), 4(c), 4(d) and 4(e) show images from medley affected site, Figs. 4(f), 4(g), 4(h) and 4(i) show images from site with inflammation and Figs. 4(j), 4(k), 4(l) and 4(m) show images from site with neoplasia. We used the low-magnification reflectance modality to navigate the surface of the excised lung sample and to identify the sites of interest. These images, shown in Figs. 4(d), 4(h) and 4(l), exhibit very different surface morphology, which indicates different states of tissue health. In Fig. 4(d), we observe normal lung parenchyma containing typical alveolar tissue, which is more difficult to discern in Fig. 4(h) and almost indistinguishable in Fig. 4(l). Corresponding low magnification H&E images from the same sites are shown in Fig. 4(c), 4(g) and 4(k), respectively. Due to typical artifacts associated with histological tissue processing, it is not possible to perfectly match the locations of these two types of images. However, by placing fiducial markers (i.e., small burn marks using a small blood vessel cauterizer) within the tissue, we are able to achieve a good correlation between the indicated areas in the low resolution H&E images (Figs. 4(c), 4(g) and 4(k)) and the corresponding reflectance images (Figs. 4(d), 4(h) and 4(l)). After acquiring...
each of the low-magnification images we switched to the multiphoton modality and acquired high-resolution multiphoton images within those regions (Figs. 4(e), 4(i) and 4(m)). Multiphoton microscopy of unstained tissues was previously shown to provide tissue diagnostics that are comparable to gold standard histology [26–28]. Furthermore, multiphoton microscopy is capable of differentiating between normal, inflammatory and neoplastic regions within mouse lung tissue [27]. Therefore, to correctly identify the health condition of the different regions within the excised tumor-laden mouse lung lobe, we compared the acquired multiphoton endomicroscopy images (Figs. 4(e), 4(i) and 4(m)) to their corresponding H&E images (Figs. 4(b), 4(f) and 4(i), respectively) and to the multiphoton microscopy images reported in ref. 27. Figure 4(e) shows a representative high-resolution multiphoton endomicroscopy image from the area indicated (red circle) in Fig. 4(d). In Fig. 4(e), mildly affected lung parenchyma is shown, where characteristic features such as alveolar walls and lumens are visible. Additionally, in this image a few alveolar macrophages may be seen within the dark-appearing alveolar lumens. Figure 4(b) shows the corresponding H&E image to Fig. 4(e) (i.e., both images are from the same tissue region) and contains similar morphological features, including intra-alveolar macrophages containing numerous eosinophilic crystals. A high-resolution multiphoton endomicroscopy image obtained within the region shown (red circle) in Fig. 4(h) is shown in Fig. 4(i). Figure 4(i) and its corresponding H&E image (Fig. 4(f)) displays a relatively large number of macrophages that have migrated and infiltrated into the alveolar lumens, thereby indicating that this region is a site of inflammation [27]. In the H&E image, the eosinophilic crystals within the macrophage cytoplasm are evident. Figure 4(m) displays a multiphoton image obtained within the area indicated (red circle) in Fig. 4(i). In Fig. 4(m) and its corresponding H&E image (Fig. 4(j)) the normal alveolar architecture is effaced by neoplastic epithelial cells forming glandular and papillary structures. These results demonstrate that our device can use the low-magnification, large FOV modality to locate tissue sites that may have different health states and then use the high-magnification multiphoton modality to closely examine these sites and obtain diagnostic quality images. Therefore, our dual modality endomicroscope is able to provide an assessment of tissue health that is comparable to standard histopathological examinations that use an optical microscope with variable magnification.
Fig. 4. **Ex vivo images of unstained tumor-laden mouse lung tissue.** (a) Low magnification image of an H&E-stained section from the periphery of the entire lung lob. The locations of the sites imaged are indicated. (b)-(e) A site that contains mildly affected lung tissue. (b) High Magnification H&E image shows mildly expanded alveolar septa surrounding lumens containing increased numbers of alveolar macrophages containing brightly eosinophilic crystals (M). (c) Low magnification H&E of the same site. (d) Low-magnification reflection/scattering image of this region within the mouse lung, in which the alveolar tissue is distinguishable. (e) High-magnification two-photon intrinsic fluorescence image of unstained ex vivo mouse lung tissue from the area displayed in (b). Alveolar lumens (A) and walls (W) are distinguishable as well as most likely a few macrophages within the lumens. (f)-(i) A site with moderate infiltrates of macrophages. (f) High magnification H&E image confirms that alveolar lumens are filled with large numbers of macrophages containing abundant intracytoplasmic eosinophilic crystals. (g) Low magnification H&E image of this site. (h) Low-magnification reflection/scattering image of this inflammatory site, in which the alveolar structure is not well distinguishable. (i) High-magnification two-photon intrinsic fluorescence image of unstained ex vivo mouse lung tissue from the area displayed in (h). A large number of cells, most likely macrophages, have migrated into the alveolar lumens, which is characteristic of inflammation. (j)-(m) A site with neoplasia proliferation of alveolar epithelial cells. (j) High magnification H&E image confirms that alveolar architecture is effaced by atypical epithelial cells forming glandular and papillary structures. (k) Low magnification H&E image of this site. (l) Low-magnification reflection/scattering image of this abnormal site, the alveolar structure is not well distinguishable. (m) High-magnification two-photon intrinsic fluorescence image of unstained ex vivo mouse lung tissue from the area displayed in (l). Compact mass of cells covers the whole area and the alveolar structure is not seen. The indicated area in (c), (g) and (k) correlates to the corresponding reflectance image in (d), (h) and (l). The red circle in (d), (h) and (l) indicates the approximate location of the site from which the multiphoton image shown in (e), (i) and (m) is obtained. Scale bars in (a), 1 mm. Scale bars in (b), (f), (j), (c), (g), (d), (h) and (l) are 100 um. Scale bars in (b), (f), (j), (c), (g), (d), (h) and (l) are 10 um.

**Mouse model.** LSL-K-ras G12D mice [29] obtained from the Mouse Models of Human Cancer Consortium were backcrossed onto a pure 129SvEv genetic background. K-ras G12D activation and Cre-mediated recombination were induced through administration of 3.16 x 10^9 viral particles of Ad-Cre virus combined with 0.144 μL 2M CaCl₂ and MEM 1x (with Earle’s salts, without L-glutamine and phenol red; Cellgro/Mediatech) to 40 μL per mouse. Adult mice were anesthetized with 2.5% Avertin intraperitoneally to effect (1μg...
tribromoethanol and 1mL tert-amyl alcohol stock solution diluted in sterile saline and filtered before use). Virus was administered intratracheally following endotracheal intubation with a 24 gauge Monoject Veterinary I.V. catheter. The mouse was euthanized and the lungs were harvested 31 weeks post viral infection. All animal housing and experimentation was performed in accordance with institutional animal care and use guidelines.

**Tissue processing.** Mice were euthanized by carbon dioxide asphyxiation. The lung lobes were removed and kept in chilled PBS until imaging. The tissue was embedded in agarose gel, plated on a standard glass microscope slide, kept immersed in PBS, and imaged within 1 hour of euthanasia. After imaging, lobes were fixed overnight in formalin and transferred to 70% ethanol before being embedded in paraffin for serial sectioning and standard hematoxylin and eosin staining for subsequent histological analyses.

3.2. *In vivo imaging*

We further demonstrated the capability of our endomicroscope by acquiring *in vivo* images of kidney (Fig. 5) from anesthetized rats without any exogenous contrast. In Fig. 5, we show low-magnification reflectance and high magnification intrinsic multiphoton fluorescence images of the rat kidney. The low magnification images show the kidney surface morphology and the fibrous components of the kidney capsule. High magnification multiphoton images were taken at various depths within the outermost regions of the kidney cortex. These images display optical cross sections of the proximal convoluted tubules, which are lined by cuboidal epithelium and separated by renal interstitium (i.e., dark, non-fluorescent spaces containing sparse amounts of connective tissue). Note that these features are clearly visible in images taken deep into the tissue (down to ~140 um beneath the kidney surface), which demonstrates that our fluorescence collection scheme consisting of large POFs is capable of endoscopic imaging deep into scattering tissues. All images were taken using ~60 mW average power at 4.1 frames/second (three frames averaged). A larger number of frame averages is possible when imaging deep because the motion artifacts (i.e., animal respiration, heart-beat, etc.) within the acquired images are largely suppressed when the endomicroscope is pressed gently against the tissue.

![Fig. 5. In vivo Images of unstained rat kidney tissues.](image-url)

(A), Low magnification reflection/scattering images of unstained in vivo rat kidney. The fibrous structure of the kidney capsule and outermost features of the kidney cortex are visible. (B)-(E), High-magnification two-photon intrinsic fluorescence image of unstained in vivo rat kidney 20 um below the surface (B), at ~60 um below the surface (C), at ~100 um below the surface (D) and at ~140 um below the surface (E). (F) High magnification H&E image shows similar features and information as two-photon intrinsic fluorescence images. (B)-(F) show cross sections of proximal convoluted tubules, each tubule contains a central lumen (CL) lined with cuboidal epithelium (CE) and separated by the poorly fluorescent renal interstitium (RI) containing sparse connective tissue components. Scale bars in (A), 100 um. Scale bars in (B)-(F), 10 um.
For the *in vivo* imaging experiments we used a normal male rat model (250-350 grams, Sprague-Dawley, Charles River Laboratories International, Inc). The rat to be imaged was placed into an induction chamber with gas anesthetic (~5% isofluorane-oxygen mixture) until achieving the sufficient level of sedation for intubation. The rat was intubated by inserting a small angiocatheter (Hallowell EMC, Rat Intubation Pack) into its trachea and extending the tube slightly past the vocal folds. A commercial otoscope (Welch Allyn Model 21700) was used to guide the angiocatheter into its proper position during this procedure. The rat was then connected to a veterinary ventilator (Hallowell EMC MicroVent 1) in order to reduce imaging artifacts due to respiration. After intubation the animal was restrained on a temperature-controlled heat pad (36° C) to maintain body temperature. The sedation (~2-3% isofluorane-oxygen mixture) was maintained through the ventilator system during the imaging session. To expose the internal organs, a small ventral-midline abdominal incision was made and each organ was isolated and elevated with tongue depressors to further reduce the motion artifacts before imaging. The endomicroscope, attached to a mechanical arm connected to a precision motorized 3D stage (MP-285, Sutter Instrument Co.), is placed on top of the organ for image acquisition. All animal treatment was in compliance with Cornell University Institutional Animal Care and Use Committee approved protocol and procedures. The average power on the sample was approximately 60 mW, frame rate of 4.1 frame/second. The pulse duration was 110 fs and the wavelength was 800 nm.

To help with endomicroscope positioning and monitoring of the animal during the imaging, an infrared imaging system was set up. The system includes an IR LED (M940L2, Thorlabs) operating at 940 nm and a CCD camera (DCU223M, Thorlabs) with a zoom lens (MVL7000, Thorlabs). The IR illumination wavelength of 940 nm does not interfere with the low magnification and high magnification imaging because of the short pass filters used (FF01-720/SP-25, Semrock) in the signal path and low PMT sensitivity at this wavelength.

### 4. Discussion

Currently, the clinical assessment of tissue health requires tissue biopsy procedures, which are guided by conventional wide field, white light endoscopes that provide a large FOV, but do not provide quality diagnostic information. Our dual modality endoscope with optical zoom capability provides, as demonstrated in the results section, high resolution diagnostic quality imaging. White light, wide field imaging naturally complements our dual modality endomicroscope. A video endoscope integrated with our device would provide imaging at three resolutions/FOVs: ~2 cm FOV with ~100 um resolution through white light imaging, ~1 mm FOV, 4.5 um resolution through the low magnification mode, and ~200 um FOV, <1 um resolution through the high magnification multiphoton imaging mode. Such multi-magnification/FOV imaging approach completely parallels the tissue biopsy procedure (i.e., white light imaging in vivo, and then dual magnification imaging of the stained biopsy specimen using a bench-top microscope ex vivo), and will allow for real time diagnostics in the clinic without tissue removal.

Maximizing the signal collection efficiency is important for a practical multiphoton endomicroscope to achieve diagnostic quality image without tissue damage. Currently, most endoscope probes are designed by optimizing the optics for excitation, and then using the same imaging optics to epi-collect the fluorescence back into the delivery fiber or to a single large core fiber [8, 17, 30]. Most biological tissues, however, strongly scatter light at the short fluorescence and second harmonic generation wavelengths, and the contribution of ballistic fluorescence photons in epi-collection diminishes quickly with increasing imaging depth [31]. Efficient collection of the scattered fluorescence photons that emerge from the tissue surface from a diffusely radiating region [32] is essential when imaging deep into a scattering tissue. By collecting the scattered fluorescence photons through 10 large core POFs, our endomicroscope improves the collection efficiency by increasing the effective collection FOV and NA; therefore, it is inherently well suited for imaging deep into highly scattering tissues.
Our current device can achieve 140 μm imaging depth without noticeable degradation of image quality (Fig. 5) and this depth limit is due to the working distance of the device. A larger working distance (e.g., ~500 μm) zoom objective lens can be achieved based on the same catadioptric concept, which will help properly evaluate the extent of tumor invasion during cancer staging [33]. Multiphoton imaging is inherently suited for deep tissue imaging, and our novel collection scheme based on harvesting non-ballistic fluorescence photons can further enhance this capability.

A future improvement for the optical zoom lens is to achieve par-focal operation, i.e., the focal planes of the two optical zooms overlap, which would eliminate the required endoscope repositioning when switching between the imaging modes/zooms. In addition, the zoom lens enables the combination of high-resolution multiphoton imaging with a variety of imaging modalities and thus may provide additional diagnostic value by combining the complementary diagnostic capabilities of several modalities in a single device. The low-magnification mode could be one-photon imaging, including any of reflectance (demonstrated in this paper), narrow-band, or laser-induced fluorescence imaging modality. The dichroic coatings and the excitation light delivery fibers can be selected in such ways that a single device can implement all these modalities by selecting the appropriate laser sources. The low-magnification mode can also be implemented through multiphoton imaging modalities because the novel, non-reciprocal fluorescence collection approach (i.e., the separation between the excitation and collection optical path) enables high collection efficiency in a low NA, large FOV excitation system.

5. Conclusion

In summary, we have designed, built, and tested, an endomicroscope that, for the first time, achieves optical zoom capability in a miniature device. Our device extends significantly the utility of high resolution endomicroscopy by combining, in the same device, the high-resolution modality with a large FOV modality. Our endomicroscope enables imaging large tissue areas with a coarse spatial resolution and locating the sites of interest, which can then be imaged with high-spatial resolution. The optical zoom operation achieved without any mechanical adjustments is enabled by a novel miniature objective lens design utilizing the principle of wavelength division multiplexing. The demonstrated endomicroscope overcomes a major limitation (i.e., small FOV) in existing high resolution endoscopic imaging, particularly in its clinical translation for real-time tissue diagnostics.

Acknowledgments

We thank members of the Xu and Webb research groups for their help. Mark Williams’s editorial help is greatly appreciated. The project was supported by National Institutes of Health/National Cancer Institute Grant R01-CA133148 and National Institutes of Health/National Institute of Biomedical Imaging and Bioengineering Grant R01-EB006736, “Development of Medical Multiphoton Microscopic Endoscopy.” RSW acknowledges support through NIH grant R01 CA108773.