Multimodal nonlinear endo-microscopy probe design for high resolution, label-free intraoperative imaging

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Abstract: We present a portable, multimodal, nonlinear endo-microscopy probe designed for intraoperative oncological imaging. Application of a four-wave mixing noise suppression scheme using dual wavelength wave plates (DWW) and a polarization-maintaining fiber improves tissue signal collection efficiency, allowing for miniaturization. The probe, with a small 14 mm transversal diameter, includes a customized miniaturized two-axis MEMS (micro-electromechanical system) raster scanning mirror and micro-optics with an illumination laser delivered by a polarization-maintaining fiber. The probe can potentially be integrated into the arms of a surgical robot, such as da Vinci robotic surgery system, due to its minimal cross sectional area. It has the ability to incorporate multiple imaging modalities including CARS (coherent anti-Stokes Raman scattering), SHG (second harmonic generation), and TPEF (two-photon excited fluorescence) in order to allow the surgeon to locate tumor cells within the context of normal stromal tissue. The resolution of the endo-microscope is experimentally determined to be 0.78 µm, a high level of accuracy for such a compact probe setup. The expected resolution of the as-built multimodal, nonlinear, endo-microscopy probe is 1 µm based on the calculation tolerance allocation using Monte-Carlo simulation. The reported probe is intended for use in laparoscopic or radical prostatectomy, including detection of tumor margins and avoidance of nerve impairment during surgery.

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References and links

1. R. Siegel, J. Ma, Z. Zou, and A. Jemal, “Cancer statistics, 2014,” CA Cancer J. Clin. 64(1), 9–29 (2014).
2. F. Di Silverio, G. D’Eramo, M. Buscarini, P. Casale, S. Di Nicola, D. Colella, and A. Sciarra, “Is there always a role for radical prostatectomy in the treatment of localized prostate cancer?” Minerva Urol. Nefrol. 47(3), 117–124 (1995).
3. V. Sharma, E. O. Olweny, P. Kapur, J. A. Cadeddu, C. G. Roehrborn, and H. Liu, “Prostate cancer detection using combined auto-fluorescence and light reflectance spectroscopy: ex vivo study of human prostates,” Biomed. Opt. Express 5(5), 1512–1529 (2014).
4. A. R. McCullough, “Sexual Dysfunction after Radical Prostatectomy,” Rev. Urol. 7(2), S3–S10 (2005).
5. O. Yossepowitch, A. Bjartell, J. A. Eastham, M. Graeven, B. D. Guillonneau, P. I. Karakiewicz, R. Montironi, and F. Montorsi, “Positive surgical margins in radical prostatectomy: outlining the problem and its long-term consequences,” Eur. Urol. 55(1), 87–99 (2009).
6. J. A. Wieder and M. S. Soloway, “Incidence, etiology, location, prevention and treatment of positive surgical margins after radical prostatectomy for prostate cancer,” J. Urol. 160(2), 299–315 (1998).
24. Y. Wu, Y. Leng, J. Xi, and X. Li, “Scanning all-fibre-optic endomicroscopy system for 3D nonlinear optical microscopy,” J. Lipid Res. 51(11), 3091–3102 (2010).

25. Y. Wu, J. Xi, M. J. Cobb, and X. Li, “Scanning all-fibre-optic endomicroscopy system for 3D nonlinear optical microscopy,” Opt. Express 21(14), 17161–17175 (2013).

26. D. R. Rivera, C. M. Brown, D. G. Ouzounov, I. Pavlova, D. Kobat, W. W. Webb, and C. Xu, “Compact and flexible raster scanning multimodal microscope,” Proc. Natl. Acad. Sci. U.S.A. 108(43), 17598–17603 (2011).

27. Y. Zhang, M. L. Akins, K. Murari, J. F. Xi, M.-J. Li, K. Luby-Phelps, M. Mahendroo, and X. D. Li, “A compact fiber-optic SHG scanning endomicroscope and its application to visualize cervical remodeling during pregnancy,” Proceedings of the National Academy of Sciences 109(32):12878–12883 (2012).

28. J. 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).

29. D. R. Rivera, C. M. Brown, D. G. Ouzounov, W. W. Webb, and C. Xu, “Use of a lensed fiber for a large-field-of-view, high-resolution, fiber-scanning microendoscope,” Opt. Lett. 37(5), 881–883 (2012).

30. D. G. Ouzounov, D. R. Rivera, W. O. Williams, J. A. Stupinski, T. L. Southard, K. H. Hume, J. Bentley, R. S. Weiss, W. W. Webb, and C. Xu, “Dual modality endomicroscope with optical zoom capability,” Biomed. Opt. Express 4(9), 1494–1503 (2013).

31. L. Fu, A. Jain, H. Xie, C. Cranfield, and M. Gu, “Nonlinear optical endoscopy based on a double-clad photonic crystal fiber and a MEMS mirror,” Opt. Express 14(3), 1027–1032 (2006).

32. L. Fu, A. Jain, C. Cranfield, H. Xie, and M. Gu, “Three-dimensional nonlinear optical endoscopy,” J. Biomed. Opt. 12(4), 045001 (2007).

33. H. Bao, J. Allen, R. Pattie, R. Vance, 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).

34. N. P. Ghimire, H. C. Bao, and M. Gu, “Broadband excitation and collection in fiber-optic nonlinear endomicroscopy,” Appl. Phys. Lett. 103(7), 073703 (2013).
One major determinant of the success of a prostatectomy procedure is disease recurrence caused by leftover malignant and premalignant tissues. However, it is difficult to directly distinguish these diseased tissues from their healthy counterparts at the tumor margin during laparoscopic or radical prostatectomy. Currently, there is no on-the-spot diagnosis technology available during prostate tumor removal surgery; the suspicious margin is taken out and sent to the lab for examination while patients remain anesthetized on the operating table. Aggressive removal of healthy tissue can lead to neurologic defects while residual malignant cancer tissue may lead to metastasis and postoperative treatment failure. Furthermore, some prostatectomy patients suffer impairment of cavernous nerves, resulting in erectile dysfunction [4–7]. Based on the need for in vivo cancer diagnosis at sub-cellular resolution for robotic surgery, a miniaturized, multimodal, nonlinear, endo-microscopy probe has been developed to identify malignant prostate tissue and tumor margin by resolving differences in cellular and subcellular structures among tissue types, without the need for optical labels or contrast agents [8, 9]. Epithelial cells, including prostate tumor cells, are rich in CH2 bonds due to abundant lipid content. CARS imaging can detect CH2 bonds with high resolution and sensitivity, therefore the label free CARS technique can be used to highlight epithelial cells, including cancer cells, within the fibrous peri-prostatic tissues. The abundant lipids within...
nerve sheaths also make an excellent target for CARS imaging. Cancer cell invasion into the surrounding tissue manipulates the existing extracellular matrix composed of arrayed collagen fibers, and these fibers provide an excellent imaging target for second harmonic generation (SHG) [16–19], which is incorporated into the capabilities of the probe.

During robotic assisted radical prostatectomy, the surgeon uses a remote manipulator interface to control the robotic arms and carry out the surgical operation through several small abdominal incisions. Minimal physical size with high imaging resolution is a key requirement for the multimodal, nonlinear endo-microscopy probe for prostatectomy as the imaging probe needs to fit into the ports of the robotic arms.

Multimodal, nonlinear endo-microscopy probe development, in its current state, has achieved fiber-delivered endo-microscopy design and modeling, but the four-wave mixing (FWM) noise caused by non-linear effects within a delivery fiber are not addressed adequately to allow for high-efficiency collection of CARS signal [20–37]. By using a polarization maintaining fiber and dual wavelength wave plate (DWW), our earlier work demonstrates that the FWM noise can be eliminated [38, 39], allowing for reduction in the size of the multimodal, nonlinear endo-microscopy probe and high signal collection efficiency. With a 14 mm transverse diameter and 1 µm image resolution, which clearly distinguishes between cellular morphology of healthy and cancerous tissue, this round-shaped, multimodal, nonlinear endo-microscopy probe has the potential for use in robotic assisted, radical prostatectomy. This work presents significant reduction of the endo-microscopy probe size without sacrificing image quality. The label-free imaging device will potentially provide surgeons and urologists a precise picture of peri-prostatic tissues, reducing the number of unnecessary biopsies and the risk of nerve impairment during the surgical procedures.

2. Materials and method

2.1 Multimodal, nonlinear endo-microscopy probe scheme

A customized micro-electromechanical systems (MEMS) scanning mirror as well as miniature optical and mechanical components are applied in the handheld, multimodal, nonlinear endo-microscopy probe. The MEMS is customized with much-reduced PCB (printed circuit board) dimensions and compact distribution of connecting electrical wires. In our design, the excitation laser (pump & stokes beam) from the fiber is collimated, reflected off the reflecting mirror, and transmitted via a 2D MEMS scanning mirror and endo-microscope onto the sample. Emission signal in the epi-direction is returned through the endo-microscope lens, MEMS, reflecting mirror, and collimator system, and is collected by a PMT (photomultiplier tube) at one end of the fiber in reverse orientation from the excitation laser. Emission signal is collected backward through the multimodal, nonlinear endo-microscopy probe. At the top surface of the tissue, epi-CARS photons are detected, while deeper in the tissue, some forward CARS photons are re-directed back towards the probe because of photons scattering.

The CARS excitation laser (Stokes and pump beams) is delivered by the polarization maintaining single mode fiber (PM1300 fiber) into the collimator system, and then the excitation laser is collimated to the parallel beam. The polarization states of the linearly polarized Stokes and pump beams are adjusted from relative orthogonal to parallel in order to suppress the four-wave mixing noise in the delivery fiber by rotating the fiber cable. The total amount of rotation of the DWW should be, at minimum, larger than 90°, which is achieved by rotating the collimator and fiber together as a whole while using the DWW as the sealing window in the MEMS frame in our system. DWW is made from quartz. Figure 1 illustrates the schematic setup of the miniaturized, multimodal, nonlinear endo-microscopy probe.
Fig. 1. The schematic optical design of the miniaturized endo-microscope of fiber-based, multimodal, nonlinear probe.

The as-built image performance is guaranteed by the modification of the spacer thickness and the adjustment of the focus position, and lens glass made from different materials is used to compensate the chromatic aberration and improve the system performance. The probe will penetrate human body tissues, so the material of the external barrel housing needs to be resistant to bodily fluid-induced corrosion. The external barrel housing is composed of stainless steel while the inner components are constructed from black anodized aluminum or ebonol-coated brass. To fit into the robotic port of a surgical robot such as the da Vinci robotics surgical system, the transverse diameter of the probe is designed to be with 15.1 mm diameter with no limit in length; for this probe, the total length is approximately 60 mm with endo-microscope approximately 6 mm in diameter and 39 mm in length.

In order to shrink the probe to fit into the port of the robotic assisted surgical system, a customized MEMS scanning mirror is incorporated. The compact circuit board is designed by AdvancedMEMS, Inc. to guarantee a miniaturized probe with the ability to image tissue in vivo [40, 41]. V grooves included in the probe can help to adjust the positional tolerance in XYZ decenter, Theta, and Phi-tilt of both the MEMS device and collimator component.

2.2 Specification and design of the multimodal nonlinear endo-microscopy probe

The endoscope component of the multimodal, nonlinear endo-microscopy probe is designed with a stainless steel housing of less than 3 mm in diameter at the distal end. Excitation laser transmission is multimodal in the 817 nm to 1064 nm wavelength range using a PM1300 HP fiber. The exit pupil diameter of the collimator is set to be 1.1 mm to match the entrance pupil size of the endo-microscope. The fiber numerical aperture (NA) is 0.12 in the 1270 nm to 1625 nm wavelength range, while the experimentally-determined effective NA dropped to 0.09 to 0.1 in the 817 nm to 1064 nm wavelength range because of the multimode laser transmission mode. Thus, the NA of the collimator has been adjusted to 0.1 to enhance the fiber and collimator coupling efficiency.
Figure 2 shows the optical imaging path of the multimodal nonlinear endo-microscopy probe. The exit pupil diameter of the collimator system is designed to match the diameter of MEMS mirror and the entrance pupil diameter of the endo-microscopy system. For the endo-microscopy part, the numerical aperture is 0.73, FOV (field of view) is 160 µm × 160 µm, and working distance is 0.37 mm. The targeted as-built resolution (rms radius) of the probe is 1 µm for FOV 100 µm × 100 µm. The total length of our CARS endo-microscopy is 40.5 mm and the apochromatic wavelengths are 450 nm, 663 nm, 817 nm, and 1064 nm. Chromatic aberration is corrected at 663 nm, 817 nm, and 1064 nm since these three wavelengths are transmitted simultaneously. The endo-microscope is designed with an apochromatic wide angle Keplerian telescope beam expander system that completely fills the back aperture of the light focusing system with collimated light. The entrance pupil of the endo-microscope is located at the vertical scanning plan of the MEMS mirror. An intermediate image is formed at the field stop of the apochromatic beam expander system. The scanning angle of the MEMS mirror is ± 7°.

The fiber cable and a dual wavelength waveplate (DWW) rotating mechanism employed for relative adjustment of the Stokes and pump beam polarization state is applied in the multimodal, nonlinear endo-microscopy probe design. The polarization state of the linearly polarized beams needs to be orthogonal in order to suppress four-wave mixing noise in the delivery fiber and the polarization is rotated to parallel by a DWW after exiting the collimating lens. To control the beam polarization arriving to the MEMS mirrors, we can rotate the fiber cable rather than the DWW plate in order to reduce the diameter of the probe. The fiber cable has 900 µm blue furcation tubing, and 2.5 mm diameter stainless steel ferrules are applied as the fiber tip connectors. The specifications of the multimodal nonlinear endo-microscopy probe are given in Table 1:

| Basic System Parameters                      | Value                                    |
|----------------------------------------------|------------------------------------------|
| CARS Excitation laser Wavelength range for delivery | 817 nm(Pump), 1064 nm(Stokes)            |
| Signal wavelength to be collected from tissue | 663 nm-CARS, 500 nm-TPEF, 400 nm-SHG     |
| Input aperture (diameter of exit pupil)      | 1.12mm (Agree with the dimension of MEMS scanning mirror) |
| Distance from the MEMS mirror to objective lens | 4 mm                                    |
| NA(after immersion in water)                  | 0.75                                     |
| As-built resolution of the probe             | 1 micron                                 |
| Resolution of the endo-microscopy part       | 0.78 µm in                               |
| FOV of the endo-microscopy probe             | FOV 160 µm × 160 µm                      |
|                                                  | 100 µm × 100 µm                          |

The nominal resolution without tolerance budget simulation of endo-microscopy is 0.46 µm resolution at the center field of view and 0.57 µm at the marginal field of view. USAF imaging experiments performed with real product of the endo-microscopy reveals the as-built resolution is 0.78 µm. For as built whole probe, including collimator and endo-microscopy, 1
µm is the resolution can be achieved according to the simulation result from Monte Carlo tolerance budget.

2.3 Tolerance budget and image performance of collimator and components of the multimodal nonlinear endo-microscopy probe

High spatial overlap on the sample image side is achieved with 0.46 µm resolution at the center of the field of view and 0.57 µm at the marginal field of view of the endo-microscope. Misalignment in the endo-microscopy system would generate mismatch between the pump and Stokes beams and create difficulty for CARS signal generation. A tolerance budget is used to ensure that each component is manufactured adhering strictly to the specifications and integrated well into the multimodal, nonlinear endo-microscopy probe system to achieve the highest resolution possible. Monte Carlo simulation of the multimodal nonlinear endo-microscopy probe was performed using the parameters listed in Table 2.

| Tolerance attribute | Radius | Airspace and glass thickness | nd | νd | Surface Irregularity | Element wedge | Element tilt | Element decenter |
|---------------------|--------|-----------------------------|----|----|---------------------|---------------|-------------|-----------------|
|                     | 0.1 mm | 0.025mm                     | 0.0005 | 0.8% | 0.25 fringe | 8 µm TIR | 0.001 radians | 20 µm |

After running the Monte Carlo tolerance budget, the as-built image performance is estimated by varying the curvature and air gap between glasses. The performance of the multimodal, nonlinear endo-microscopy probe is: (1) the MTF (modulation transfer function) is larger than 0.2 @ 640 line pairs/mm, and (2) the RMS spot radius, which showed the resolution is smaller than 1 µm within 98% field of view. The image resolution is guaranteed within 1 µm in our as-built probe. Precise assembly and performance testing is needed to ensure each component is manufactured in strict adherence to specifications and integrated into the endo-microscope system within the specified tolerance budget. The as-built image performance of the multimodal, nonlinear endo-microscopy probe is shown in Fig. 3. Figure 3(a) showed the MTF performance of the as-built multimodal nonlinear endo-microscopy probe. Figure 3(b) showed the spot diameter/ resolution at different FOVs of the multimodal nonlinear endo-microscopy probe. MTF and spot diameter are used as performance indicators in the as-built image performance simulation. If each component of the probe adheres strictly to the specified tolerance budget, the resolution of the multimodal nonlinear endo-microscopy probe will be within 1 µm in 98% field of view with 90% probability.
3. Experimental setup and results

3.1 Testing of endo-microscope

Figure 4(a) and 4(b) present the manufactured product of the CARS collimator and fiber cable with 900 μm blue furcation tubing and 2.5 mm diameter stainless steel ferrules. The wavefront quality of the fiber collimator was evaluated at 785 nm using an Optocraft wavefront sensor. The airspace between the fiber and optics was adjusted to achieve...
wavefront quality smaller than 0.05 λ RMS at 785 nm, which is within the design tolerance 0.5 λ at 785 nm. Figure 4(c) showed the multimodal, nonlinear endo-microscopy probe lens.

The experimental setup for the endo-microscopy resolution testing is shown in Fig. 4(d), where the resolving power of the endo-microscopy lens was investigated by acquiring transmission images of a USAF resolution test target (Edmund Optics). The USAF resolution target was placed at the focus position of the endo-microscopy barrel. The 20 mm objective lens and a Samsung cell phone image receiver were used to acquire high resolution targeted images. Light of different wavelengths (575 nm, 660 nm, and 850 nm) was used to test the resolution and the results are shown in the Fig. 4(e), 4(f), and 4(g) respectively. The ‘3’ in the ninth group is the smallest element resolved by the endo-microscope with a spacing of 645 line pairs/mm, corresponding to a line width of approximately 0.78 μm. There is no blurriness in the shape of the individual lines in the image except for a conical defocus deformation in the 660 nm resolution image in which the transmission resolution image is not at the center of the objective.

![Image](https://example.com/image.png)

**Fig. 4.** (a). Photo shows PM1300 fiber part of the multimodal, nonlinear endo-microscopy probe; (b) Photo shows the collimator part of the multimodal, nonlinear endo-microscopy probe; (c) Photo shows the endo-microscopy part of the multimodal, nonlinear endo-microscopy probe; (d) resolution experiment setup of the endo-microscope; (e), (f), and (g) present the transmission images in 575 nm (e), 660 nm (f), and 850nm (g) from endo-microscopy imaging of USAF resolution target.

### 3.2 Multi-modal prostate cancer tissue imaging

To demonstrate the potential utility of CARS and SHG imaging for prostatectomy procedures, we imaged prostate tissue with an upright microscope. Human prostate tissues were obtained from the Houston Methodist Hospital’s Tissue Bank. These specimens, obtained from patients undergoing radical prostatectomy, were snap-frozen immediately after tissue removal and thawed the tissues at room temperature approximately 30 min before imaging. Figure 5 illustrates an example of the CARS and second harmonic generation (SHG) imaging result for prostate glands and the surrounding stroma with an upright microscope setup.

For CARS imaging, we used 817 nm and 1064 nm as pump and Stokes wavelengths to match the vibrational frequency of CH2 bond at 2845 cm⁻¹ and collect CARS emission at 640-680 nm. Cell nuclei are shown as dark round spots in Fig. 5(a) (yellow arrow). Based on earlier experimental results from our group, differentiation of cancerous glands from normal prostate tissue can be determined with real-time CARS imaging through cellular features (cell neighbor distance as well as size and shape of nuclei). Type I collagen fibers in stroma are shown by SHG signals collected at 405-415 nm (Fig. 5(b)). Combining the two modalities of CARS and SHG, cancer cell invasion into surrounding stroma can be visualized, which is critical for the detection of prostate cancer cells at a surgical margin. To incorporate the multi-
modal, label-free imaging technology into a real-time diagnostic platform, the size of the miniaturized optics is a critical factor as the fiber probe has to fit into ports of robotic arms and penetrate the incisions in the patient’s abdomen. Resolution is the other critical factor essential to obtain clear visualization of cell nuclei with sizes around 5-10 μm. With an estimated resolution of 1 μm, we expect to detect and differentiate normal and diseased prostate glands and stroma based on cellular feature analysis at the surgical margin.

The representative SHG and CARS images for prostate glands are showed in Fig. 5. Our ongoing work includes combination of previous and current SHG and CARS imaging platform and endo-microscopy, which is used in place of the upright, microscope for tissue imaging in real time on-site cancer diagnosis. We expect that the label-free multimodal nonlinear endo-microscope can potentially be applied to video-rate in vivo tissue imaging during intraoperative or interventional procedures and save precious time in sending tissue specimens for sectioning, staining, and diagnosis in a pathology laboratory [42–44].

4. Discussion and conclusion

Using a sophisticated CARS microscope bench to achieve high image resolution, a FWM noise suppression scheme to eliminate the FWM noise in the delivery fiber, and miniaturized CARS endo-microscope with high definition, a CARS probe proof of concept is demonstrated, paving the path towards ultimate integration of a compact fiber laser with a miniaturized handheld multimodal CARS probe for minimally-invasive, label-free, intraoperative imaging.

The design and performance of a prototype high resolution, label-free, multimodal, nonlinear endo-microscopy probe is presented in this paper. The transmission images of the smallest bars in Group 9, Element 3 on a USAF1951 target are obtained with the endo-microscope at 575 nm, 660 nm, and 850 nm separately, which present resolutions of endo-microscopy reaching 0.78 μm at these wavelengths. Image testing results show that the multimodal, nonlinear endo-microscope probe meets the design specifications derived from computational modeling with good optical alignment, and at the same time, unwanted fiber-generated non-resonant FWM noise is eliminated using the applied polarization scheme.

In addition, we demonstrate a 15.1 mm transverse diameter round-shaped multimodal nonlinear endo-microscopy probe, with 1 μm as-built resolution image performance according to Monte-Carlo simulation, to enable handheld multimodal imaging for real-time, minimally-invasive prostate cancer diagnosis. A fiber laser could be used to replace the tabletop
Ti:sapphire laser, making the entire multimodal imaging setup compact enough to be rolled into the operating room for use in laparoscopic or radical prostatectomy, including detecting tumor margins and avoiding nerve impairment during surgery, ultimately improving the outcomes of the prostatectomy.

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