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14. ABSTRACT
Direct visualization of cavernous nerves (CNs) and prostate surgical margins can improve nerve preservation and post-operative potency of radical prostatectomy. Coherent Anti-Stokes Raman scattering (CARS) is a label-free imaging technique which can differentiate prostate cancer tissues and cavernous nerves (CNs) without the use of any labeling agents. However, one major obstacle for intraoperative application of CARS is the bulky size of the original CARS system which was built on free space optics. In previous calendar years, we have successfully refined our original bulky CARS system into a compact fiber-based microendoscope device and finished the development of a miniaturized fiber probe. We have also tested the feasibility of using TRAMP mice for prostate surgical margin imaging. The next step will be *in vivo* imaging and algorithm development with the all-fiber microendoscope system. This work presents a significant milestone in CARS endoscopy development using micro optics to greatly reduce the size of CARS endoscopy while keeping high imaging definition quality.

15. SUBJECT TERMS
coherent anti-Stokes Raman scattering; endoscopy; endomicroscopy; fiber optics; micro-electromechanical systems

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1. Introduction

Direct visualization of cavernous nerves (CNs) and prostate surgical margins can improve nerve preservation and post-operative potency of radical prostatectomy. Coherent Anti-Stokes Raman scattering (CARS) is a label-free imaging technique which can differentiate prostate cancer tissues and cavernous nerves (CNs) based on intrinsic macromolecular contrast without the use of any labeling agents. Our goal is to develop a miniaturized Coherent Anti-Stokes Raman scattering (CARS) system which is more suitable for intraoperative applications. In previous calendar years, we have replaced the bulky free space optics with their fiber optic counterparts to build a compact fiber-based microendoscope device. We are currently working on the assembly and characterization of the fiber-based microendoscope system and will complete the microendoscope evaluation on patient specimen and animal models.

2. Keywords

Coherent anti-Stokes Raman scattering; non-linear optics; endomicroscopy; fiber optics; micro-electromechanical systems; prostate cancer; prostatectomy, *in vivo* cancer differentiation; optical diagnostics; medical imaging; image quantification; machine learning

3. Overall Project Summary

The specific aims have not been significantly modified from the original approved statement of work. Aim 1 is to develop a miniaturized CARS microendoscope by refining the original bulky CARS system into a compact all-fiber device. Aim 2 is to evaluate the CARS microendoscope for cavernous nerve and prostate surgical margin imaging.

Aim 1: To refine our existing CARS microendoscope into a fiber device for *in vivo* cancer imaging.

A. Refine laser, detection, and data acquisition (DAQ) subsystems with fiber optic components.

B. Develop a fiber probe based on microelectromechanical systems (MEMS) technology and consisting of a polarization maintaining fiber, a MEMS scanning mirror, and micro-optics.

C. Assemble and characterize the all-fiber-based CARS microendoscope system.

We have refined our original bulky free-space optical CARS system into a compact all-fiber CARS microendoscope and finished the development of the fiber probe (Aim 1A and Aim 1B) in previous calendar years. However, the probe fabrication was delayed by the vendor for a year and we have just received the probe in mid July 2015. We had requested a twelve-month no cost extension and the new ending date of Sep 29 2016 has been approved. We are currently working on the assembly and characterization of the miniaturized fiber-based microendoscope.

Aim 2: To evaluate the ability of our CARS microendoscope to image cavernous nerves and prostate surgical margins *in vivo* using intrinsic CH_2-based molecular contrast.

A. Evaluate the utility of the CARS microendoscope to image patient surgical specimens ex vivo and transgenic mouse model for prostate cancer (TRAMP) mice in vivo.

We have tested the feasibility of using TRAMP mice for prostate surgical margin imaging and has reported the results in the annual report of 2014.

B. Develop algorithms for image quantification and characterization of prostate and periprostatic tissues in TRAMP mice.

C. Validate the performance of microendoscope imaging and image analysis algorithms to identify prostate and periprostatic tissue in vivo.

We will complete the Aim 2B and Aim 2C in the no-cost extension year. We will first test the CARS probe on fresh patient tissue obtained from Houston Methodist Hospital. We expect that cell and tissue structures can be visualized by our fiber-based CARS system. In vivo imaging studies will be carried out with TRAMP mice for the differentiation of prostate cancer, tumor margin, and normal tissues. Sprague-Dawley rats will be used for imaging of Cavernous Nerves. A computerized image analysis algorithm will then be developed to differentiate cancer from non-cancer samples for real-time diagnosis. The accuracy of the tissue and nerve classification algorithm will be estimated on independent test sets of patient specimen and animal subjects.

4. Key Research Accomplishments

1. Fabrication of the miniaturized CARS microendoscope probe
2. Assembly and characterization of the miniaturized fiber-based microendoscope system

5. Conclusion

We have successfully finished the fabrication of a miniaturized fiber-optic-based CARS microendoscope probe. The 14 mm transversal diameter of the round-shaped probe paves the path towards a future handheld multimodal CARS imaging for real-time intraoperative imaging. We have examined the feasibility of multimodal prostate imaging with CARS and SHG using patient specimens and mouse models. We are currently working on the assembly and characterization of the fiber-based microendoscope system. We will test the application of all-fiber microendoscope system on fresh patient tissue and live animal models in the no-cost extension year. We will also develop a computerized image analysis algorithm for real-time diagnosis.

6. Publication, Abstracts, and Presentations

Nothing to report

7. Inventions, Patents, and Licenses

Nothing to report

8. Reportable Outcomes

1) A miniaturized CARS probe has been fabricated for *in vivo* identification of cavernous nerves and differentiation of prostate cancer and surgical margins.

2) The miniaturized fiber-based microendoscope system is being assembled and tested for potential intraoperative imaging.

9. Other Achievements

1) This DOD award supports two postdoctoral trainees (Xu Chen and Xiaoyun Xu)

2) This award also provides research opportunities for one PhD student (Sheng Weng)

10. References

1. Chen X, Xu X, McCormick DT, Wong K, Wong ST. Multimodal nonlinear endo-microscopy probe design for high resolution, label-free intraoperative imaging. Biomed Opt Express. 2015 Jun 3;6(7):2283-93.