Scheme for efficient fiber-based CARS probe

Mihaela Balu[*1], Gangjun Liu[*1], Zhongping Chen[1,2], Bruce J. Tromberg[1,2], and Eric O. Potma[1,3]

[1] *Laser Microbeam and Medical Program (LAMMP), Beckman Laser Institute and Medical Clinic, 1002 Health Sciences Road East, University of California, Irvine, 92697*
[2] *Department of Biomedical Engineering, University of California, Irvine, 92697*
[3] *Department of Chemistry, University of California, Irvine, 92697*
[*] *contributed equally to this work*
z2chen@uci.edu; epotma@uci.edu

Abstract. We demonstrate a fiber-based probe for maximum collection of the Coherent anti-Stokes Raman Scattering (CARS) signal in biological tissues. We discuss the design challenges including capturing the back-scattered forward generated CARS signal in the sample and the effects of fiber nonlinearities on the propagating pulses. Three different biological tissues were imaged *in vitro* in order to assess the performance of our fiber-delivered probe for CARS imaging, a tool which we consider an important advance towards label-free, *in vivo* probing of superficial tissues.

1. Introduction

Making new optical technologies available to patients requires the translation of concepts developed in the laboratory to compact devices suitable for use in clinical settings. Recent advances in this direction include development of fiber-based compact probes which are being used successfully in optical imaging techniques based on optical coherence tomography[1], two-photon fluorescence (TPEF) and second harmonic generation (SHG)[2,3]. Among the optical imaging methods currently used, the development of coherent anti-Stokes Raman Scattering (CARS) remains at a level much closer to the laboratory bench than to the clinic. Integrating CARS with fiber delivered probes would open the way for label-free, *in vivo* probing of superficial tissues.

The challenges for developing a CARS probe include efficient collection of the backward scattered signal generated in the sample into the fiber for detection and the effects of fiber nonlinearities on the propagation pulses. We investigated self-phase modulation, stimulated Raman scattering (SRS) and four-wave-mixing (FWM) generation in the fiber: nonlinear processes expected to occur in a two-beam excitation based probe.

In previous works, single mode fibers have been successfully used in initial designs of CARS fiber probes.[4] Compared to single mode fibers (SMF), the use of photonic crystal fibers (PCF) is more attractive because they can be optimized for the delivery of both picosecond and femtosecond pulse trains. PCFs are thus an excellent design choice for the development of fiber-based multimodal CARS microscopy, which incorporates TPEF and SHG modalities that often necessitate the higher pulse energy of femtosecond pulses.

Multiphoton Microscopy in the Biomedical Sciences X, edited by Ammasi Periasamy, Peter T. C. So, Karsten König, Proc. of SPIE Vol. 7569, 75693A · © 2010 SPIE · CCC code: 1605-7422/10/$18 · doi: 10.1117/12.855187

PCFs have been used for delivering picosecond pump and Stokes pulses to the sample[5], but successful detection of CARS signals through a PCF has yet to be demonstrated.

In this work, we advance the development of a CARS fiber probe by carefully optimizing the pulse delivery and the signal collection efficiencies of the fiber.

2. Experimental design and results

To optimize the design of the fiber delivered probe, we first tested three different single mode fibers in terms of laser pulse delivery: a single mode fused silica (SMF, 40 cm long, 4.8 μm core diameter), a double-clad photonic crystal fiber (DCPCF, 80 cm long, 16 μm core diameter) and a large mode area photonic crystal fiber (LMA PCF, 50 cm long, 20 μm core diameter).

Femtosecond pulses from a tunable Ti:Sapphire oscillator were used for the pump beam in the CARS process. The Stokes beam was derived from a 1064 nm picosecond laser source. The two laser sources were synchronized using an active feedback scheme. For the experiments presented in this work, the femtosecond source was tuned to 817 nm, corresponding to a wavenumber of 12240 cm^{-1}, resulting in a 2842 cm^{-1} shift between the pump and Stokes pulses, which matches the Raman shift of the CH_2 stretch in lipids.

Pulse delivery without significant spectral and/or temporal broadening is an important criterion for selecting a delivery fiber. Although it has been shown that pulse broadening effects are minimum in standard silica SMFs with lengths of less than 1m for picosecond pump and probe pulses with energies of a few nJ^4, such fibers do not support femtosecond pulse trains with pulse energies relevant to CARS microscopy. To avoid spectral broadening effects, PCFs have been used as the primary excitation delivery fibers in multiphoton microscopy.[6, 7]

We observed limited spectral broadening of the femtosecond and picosecond pulses in both large mode area and double-clad PCFs of length less than 1 m. While the spectral broadening was not a concern for the individual pump and Stokes pulses in the PCF fibers, an important finding in this work was that for all the fibers tested, a strong four-wave-mixing (FWM) contribution at the anti-Stokes frequency was generated in the delivery fiber under typical CARS excitation conditions. The spectral content of the anti-Stokes shifted radiation is shown in Figure 1. This fiber-generated FWM component forms a large background that overwhelms the signal generated in the sample, and severely complicates the interpretation of the image unless removed.

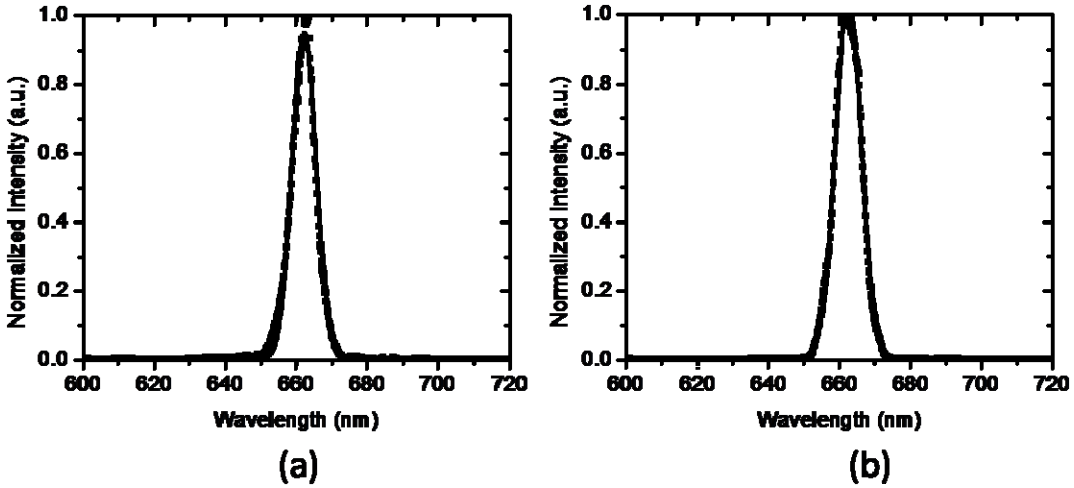

Figure 1. Spectrally-resolved anti-Stokes four-wave-mixing signal measured at the output of (a) the LMA20 fiber (FWHM=7.9 nm) output and (b) the silica SMF (FWHM=8.6 nm).

The isolated anti-Stokes component shows a well-defined spectral profile that corresponds to the spectral convolution of the pump and Stokes pulse spectra. Because no additional broadening of this shifted component is observed, we conclude that this contribution is generated directly through a nonlinear mixing process between the pump and the Stokes pulses, and thus independent of the SPM mechanism. Importantly, we observed an identical anti-Stokes component in the case of the silica SMF, confirming that the anti-Stokes shifted this component is not the result of accidental phase-matching in the PCF fiber. We verified that the intensity of the anti-Stokes component scales quadratically with the pump light and linearly with the Stokes radiation, confirming that this shifted contribution is the result of a four-wave-mixing process.

The presence of intrinsic anti-Stokes generation in the fiber necessitated spectral filtering of the excitation light before focusing it into the sample. We employ a simple scheme for removing the FWM signal contribution. Given the observed fiber nonlineartities, an LMA PCF was chosen due to its favorable dispersion properties relative to a standard single mode optical fiber. A separate fiber for collection of the signal was implemented. Therefore, the probe design incorporates a delivery fiber, a dichroic mirror for filtering out the anti-Stokes signal generated in the fiber, scanning optics, an objective lens and a separate multi-mode fiber for efficient signal collection. The experimental set-up is described in Figure 2.

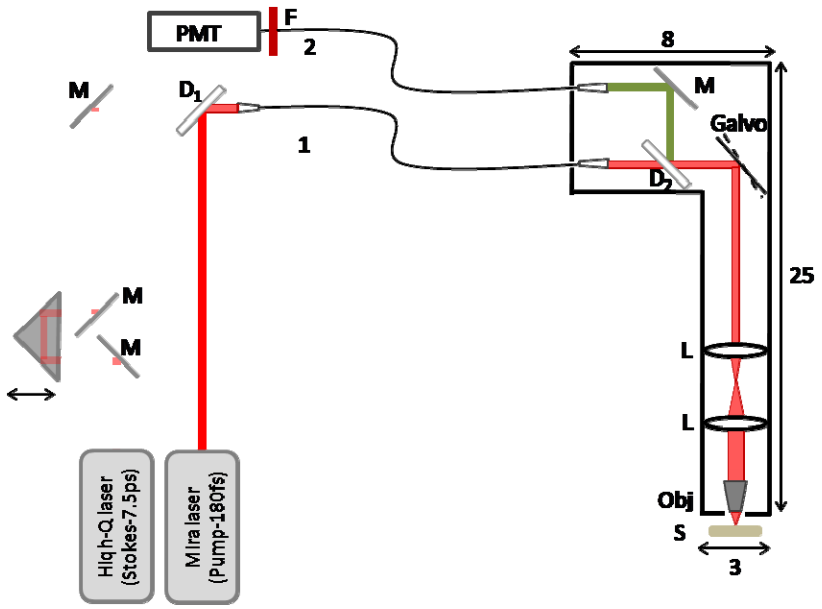

Figure 2. Schematic diagram of the fiber-delivered probe for CARS tissue imaging: M-mirrors; D_1- 1000 nm longpass dichroic mirror; D_2- 760 nm longpass dichroic mirrors, L-lens; Obj-objective; S-sample; F- 670 nm bandpass filter. Fiber 1 is used for delivery of the excitation pulses and fiber 2 is used for detecting the CARS radiation. The dimensions of the probe are indicated in cm.

To evaluate the performance of our fiber-delivered probe for CARS imaging, we imaged three different biological tissues *ex vivo*. We chose to take images of skin and eyelid, superficial tissues that would be easy to access in future *in-vivo* imaging. Therefore, we imaged adipocytes in freshly excised samples of mouse ear (CD1/C57 black wild type mice – Fig. 3a) and rabbit skin (Pathogen-free New Zealand White rabbits Oregon Rabbit Supply – Fig. 3b). In addition, the lipid content in the Meibomian gland of a 2-month old mouse was examined (Fig. 3c).

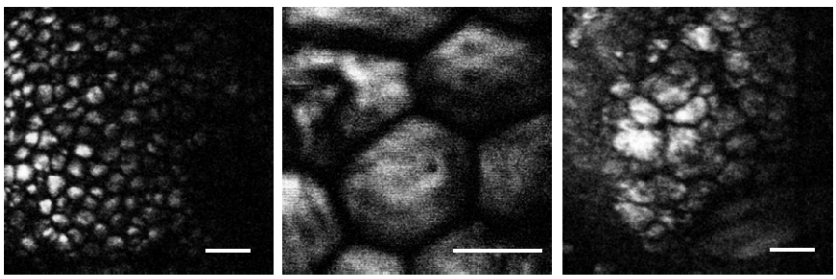

Figure 3. CARS images of thick tissue samples *ex vivo* at 2842 cm^{-1} a) Small adipocytes of mouse ear skin. b) Adipocytes of subcutaneous layer of rabbit skin tissue. c) Meibomian gland in mouse eyelid. Images were acquired in 2s. Scale bar is 50 μm.

The contrast observed is comparable to the contrast seen in CARS images obtained through free-space detection of the back-scattered light.[8] This suggests that the detected signal includes the back-scattered, forward generated CARS radiation, and that the contrast is not dominated by aperture effects at the detection fiber. In addition, the contrast is not affected by spurious anti-Stokes components from the delivery fiber, resulting in images that originate solely from CARS generation in the tissue.

3. Conclusion

We have demonstrated a fiber-delivered probe suitable for CARS imaging of thick tissues. Our design is based on two advances. First, we identified that a major problem in CARS probe design is the presence of a very strong anti-Stokes component in silica delivery fibers generated through a FWM process. Without proper spectral filtering, this component may affect the CARS image from the tissue sample. Our scheme efficiently suppresses this spurious anti-Stokes component through the use of a separate fiber for excitation delivery and for signal detection, which allows the incorporation of dichroic optics for anti-Stokes rejection. Second, we optimized the detection of backscattered CARS radiation from the sample by using a large core multimode fiber in the detection channel. This scheme produces high quality CARS images free of detector aperture effects. We expect that further miniaturization of this fiber-delivered probe will result in a handheld probe for clinical CARS imaging.

4. Acknowledgments

This work was supported by the National Institutes of Health (NIH) (EB-00293, CA-91717, RR- 01192), National Science Foundation (NSF) (BES- 86924), Air Force Office of Scientific Research (AFOSR) (FA9550-04-0101), and the Beckman Laser Institute Endowment.

References

1. W. Jung, D. T. McCormic, Y. C. Ahn, A. Sepehr, M. Brener, B. Wong, N.C. Tien, and Z. Chen, “In vivo three-dimensional spectral domain endoscopic optical coherence tomography using a microelectromechanical system mirror”, Opt. Lett. **15,** 3239-3241 (2007).
2. W. Jung, S. Tang, D. T. McCormic, T. Xie, Y. C. Ahn, I. V. Tomov, T. B. Krasieva, B. J. Tromberg, and Z. Chen, "Miniaturized probe based on microelectromechanical system mirror for multiphoton microscopy," Opt. Lett. **33**, 1324-1326 (2008).
3. G. 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**, 2249-2252 (2009).
4. F. Légaré, C. L. Evans, F. Ganikhanov, and X. S. Xie, "Towards CARS endoscopy," Opt. Express **14**, 4427-4432 (2006).

5. H. Wang, T. B. Huff, and J. X. Cheng, "Coherent anti-Stokes Raman scattering imaging with a laser source delivered by a photonic crystal fiber," Opt. Lett. **31**, 1417-1419 (2006).
6. D. G. Ouzounov, K. D. Moll, M. A. Foster, W. R. Zipfel, W. W. Webb, and A. L. Gaeta, "Delivery of nanojoule femtosecond pulses through large-core microstructured fibers," Opt. Lett. **27**, 1513-1515 (2002).
7. W. Göbel, A. Nimmerjahn, and F. Helmchen, "Distortion-free delivery of nanojoule femtosecond pulses from a To:sapphire laser through a hollow-core photonic crystal fiber " Opt. Lett. **29**, 1285-1287 (2004).
8. C. L. Evans, E. O. Potma, M. Puoris'haag, D. Cote, C. Lin, and X. S. Xie, "Chemical imaging of tissue *in vivo* with video-rate coherent anti-Stokes Raman scattering (CARS) microscopy," Proc. Natl. Acad. Sci. USA **102**, 16807-16812 (2005).