Second-harmonic optical coherence tomography

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Received November 8, 2003

Second-harmonic optical coherence tomography, which uses coherence gating of second-order nonlinear optical responses of biological tissues for imaging, is described and demonstrated. Femtosecond laser pulses were used to excite second-harmonic waves from collagen harvested from rat tail tendon and a reference nonlinear crystal. Second-harmonic interference fringe signals were detected and used for image construction. Because of the strong dependence of second-harmonic generation on molecular and tissue structures, this technique imparts contrast and resolution enhancement to conventional optical coherence tomography. © 2004 Optical Society of America

OCIS codes: 170.4500, 170.3880, 190.4160.

Optical coherence tomography (OCT) is a noninvasive, noncontact imaging modality for cross-sectional imaging of biological tissue with micrometer-scale resolution. OCT uses coherence gating of backscattered light for tomographic imaging of tissue structures. Variations in the light-scattering properties of tissue that are due to inhomogeneities in the optical refractive index provide imaging contrast. In many instances and especially in the early stages of disease, changes in tissue scattering properties between normal and diseased tissue are small and difficult to measure. To enhance the image contrast of OCT, a number of extensions have been developed: Optical Doppler tomography combines the Doppler principle with OCT to obtain tissue structural images and measure blood flow simultaneously; spectroscopic OCT combines spectroscopic analysis with OCT to obtain depth-resolved tissue absorption spectra; polarization-sensitive OCT combines polarization sensitive detection with OCT to determine tissue birefringence. Recently, engineered microsphere contrast agents for OCT were developed. Coherent anti-Stokes Raman scattering interferometry has also been reported, but no tomographic image has also been demonstrated.

Optical second-harmonic generation (SHG) is the lowest-order nonlinear optical process in which second-order nonlinear optical susceptibility is responsible for the generation of light at the second-harmonic (SH) frequency. Because second-order nonlinear optical susceptibility is determined by detailed electronic configurations, molecular structures and symmetries, local morphologies, and ultrastructures, using SHG for biomedical imaging can give unique information regarding tissue structure symmetry. The quadratic power dependence of SHG on the refractive index provides greater optical contrast for imaging tissue structures than conventional linear reflectance microscopy does. A SHG signal is typically detected in transmission mode for a bulk transparent medium. However, detection of SHG signal in reflection mode has been widely used to study nonlinear effects at surfaces and interfaces. Recently, backreflected SHG signals from unstained biological tissues were investigated and used for imaging. Quantitative SHG microscopy in collagen has also been reported. The use of SHG interference to determine molecular orientation has also been demonstrated.

In this Letter we demonstrate what is to our knowledge the first second-harmonic optical coherence tomography (SH OCT). SH OCT combines the sample structural sensitivity of SHG with the coherence gating of OCT. The system consists of an interferometer illuminated by a low-coherence light source. If the sample possesses certain structures that lack a center of symmetry, the incoming light is converted into SH waves at the sample site. SH waves are also produced in the reference arm through a nonlinear crystal. The temporal interference pattern of these SH waves is then detected and used for image construction. Because of coherence gating, the depth resolution of imaging is determined by the coherence length of the SH wave and is independent of the objective. Thus, high depth resolution is achievable even when a low-numerical-aperture objective is used. SH OCT also provides considerable imaging contrast and resolution enhancement compared with conventional OCT because of the high selectivity of SHG on tissue molecular structure and shorter measuring wavelength.

The experimental configuration of a SH OCT system is shown in Fig. 1. The light source was a mode-locked femtosecond Ti:sapphire laser centered at 800 nm with a 110-fs pulse duration and a 76-MHz repetition rate. A long-wave pass filter (F1) filtered out the spurious SHG produced by the laser. A polarizing beam splitter split the input beam into the two arms of the interferometer, and the split ratio was controlled by a half-wave plate (HWP2). In the reference arm a 0.1-mm-thick nonlinear crystal of β-barium borate was oriented for type I phase matching to convert the fundamental wave into a SH wave at 400 nm. An important requirement for the nonlinear crystal in the sample arm is that it be phase matched for the whole spectrum of the fundamental radiation. A moving mirror (M2) acted as the delay line. In the signal arm the fundamental wave was focused onto the sample by a low-numerical-aperture objective (L: numerical aperture, 0.2; f = 31.8 mm). When the sample had second-order nonlinear properties, the fundamental wave generated a SH wave.
Backreflected fundamental and SH waves were collimated by the same objective and directed by a dichroic beam splitter toward a combining beam splitter (BS2). The dichroic beam splitter reflected most of the SH wave and ~5% of the fundamental wave. The waves from the two arms were recombined after they passed through BS2. Changing the optical path delay in the reference arm caused the pulses to overlap temporally and generate interference fringes at SH and fundamental wavelengths. With proper filtration provided by filters F2, F3, and F4, the SH and fundamental interference fringe signals were detected by a photomultiplier tube and a photodiode, respectively. A prism pair was inserted into the signal arm to compensate for the group-velocity dispersion of the fundamental and SH waves, thus facilitating simultaneous detection of SH OCT and conventional OCT signals.

To measure the coherence lengths in this hybrid OCT system we replaced the sample with a polished GaAs crystal to produce surface SH waves (a nonlinear optical mirror).\(^7,12\) Interference at both wavelengths was measured as shown in Fig. 2, where SH interference occurs at exactly double the frequency of the fundamental interference. It is well known that for a Gaussian pulse \(I(t) \sim \exp[-(2t/\tau_p)^2 \ln 2]\) with pulse duration \(\tau_p\) and that coherence length \(l_c\) is given by \(l_c = (2 \ln 2/\pi)(\lambda_0^2/\Delta \lambda)\), where \(\lambda_0\) is the center wavelength and \(\Delta \lambda = (2 \ln 2/\pi)(\lambda_0^2/c \tau_p)\) is the spectral width (full width at half-maximum). The relationships \(\Delta \lambda_1/\Delta \lambda_2 = 4/\sqrt{2}\) and \(l_{c1}/l_{c2} = \sqrt{2}\) can be obtained from simple calculations, where \(\Delta \lambda_1\) and \(\Delta \lambda_2\) are the spectral widths and \(l_{c1}\) and \(l_{c2}\) are the coherence lengths of the fundamental and the SH waves, respectively. The fundamental laser radiation has a spectral width of 8.1 nm (Fig. 3A). The spectral width of the SH wave from the \(\beta\)-barium borate crystal was 3.0 nm (Fig. 3B). The coherence lengths of the fundamental and the SH waves in free space were 33 \(\mu\)m (Fig. 3C) and 24 \(\mu\)m (Fig. 3D), respectively. All measured values are in good agreement with theoretical predictions.

The sample used in our study was type I collagen harvested from rat tail tendon.\(^8\) The sample consisted of two ~30-\(\mu\)m-thick collagen layers sandwiched among three 170-\(\mu\)m-thick glass slides (schematic shown in Fig. 4, top). The Gaussian beam approximation yielded a depth of focus of 0.52 mm at the objective. The average excitation power entering the objective was approximately 50 mW, and the estimated peak power density at the beam waist in the sample was ~3.2 GW/cm\(^2\). This peak intensity is 2 orders of magnitude smaller than the peak intensity threshold for the loss in cell viability demonstrated by König et al.\(^13\) We conducted the tomographic imaging experiment by scanning mirror M2 in the delay line and recording the fundamental and the SH interference signals with a lock-in amplifier. The lock-in amplifier demodulated the interference fringe.
envelope signal with high sensitivity and precision. The measured OCT signals of one typical depth (z direction) scan are shown in Fig. 4. The conventional fundamental OCT signal in Fig. 4A shows the sandwich structure of the sample. The strong reflectance that occurs at the first air–glass interface suppresses signals from the deeper layers. The SH OCT signal in Fig. 4B shows two peaks that correspond to the two-layer structure, as SH signals are produced only in the two collagen layers. Comparison of Figs. 4A and 4B shows that there is no SH OCT signal coming from the air–glass interface, indicating that SH OCT provides good contrast for nonlinear media. SH OCT signals reveal information regarding the second-order nonlinear properties of the sample that cannot be provided by conventional OCT signals. Figure 5 is a SH OCT image of the center part of the sample, where two layers of collagen can be clearly observed.

SHG serves as a unique contrasting mechanism for tissue structure because SH signals are highly dependent on the orientation, birefringence, and local symmetry properties of the tissue. SHG efficiency in the collagen sample depends on orientation of collagen fibrils relative to the incident electrical field polarizations. In the experiment, half-wave plate HWP3 was used to control the input beam’s polarization to the sample. Another half-wave plate optimized for the SH wavelength can be inserted into the reference arm after the reference crystal. By matching the SH wave’s polarization in both arms one can highlight collagen fibrils with different orientations preferentially to produce polarization-dependent tomographic images.

In conclusion, we have presented a noninvasive optical tomography technique, second-harmonic optical coherence tomography and experimentally demonstrated the feasibility of using this technique to image biological samples. SH OCT enhances image contrast and resolution of conventional OCT by using the same light source, which extends the capability of conventional OCT to detect small changes in tissue and in molecular structure and symmetry. This technique can also be extended to third-harmonic generation and other coherent nonlinear processes for tomographic imaging.

This research was supported by research grants awarded by the National Science Foundation (BES-86924) and the National Institutes of Health (EB-00293, NCI-91717, and RR-01192). Institute support from the U.S. Air Force Office of Scientific Research (F49620-00-1-0371) and the Beckman Laser Institute Endowment is also gratefully acknowledged. Z. Chen’s e-mail address is zchen@laser.bli.uci.edu.

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