Nonlinear scanning laser microscopy by third harmonic generation

Y. Barad,[a] H. Eisenberg, M. Horowitz, and Y. Silberberg
Department of Physics of Complex Systems, The Weizmann Institute of Science, Rehovot 76100, Israel

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Third harmonic generation near the focal point of a tightly focused beam is used to probe microscopical structures of transparent samples. It is shown that this method can resolve interfaces and inhomogeneities with axial resolution comparable to the confocal length of the beam. Using 120 fs pulses at 1.5 μm, we were able to resolve interfaces with a resolution of 1.2 μm. Two-dimensional cross-sectional images have also been produced. © *1997 American Institute of Physics.* [S0003-6951(97)03408-6]

The observation of transparent samples has always been a challenge in optical microscopy, a challenge that led to several classic inventions in optics, such as phase contrast and differential interference microscopy. These methods convert phase differences due to spatial variations in refractive index to observable intensity difference. In this letter we propose a method for observation of transparent objects through variations in their nonlinear properties; third-order nonlinear effects and specifically third harmonic generation (THG) are particularly suitable for this purpose since all materials have nonvanishing third-order coefficient. THG is a well understood and characterized nonlinear effect, but it has found only limited applications due to the high light intensities that are typically required. The advances in ultrafast laser technology enable now to reach high peak powers with relatively inexpensive, low-average-power lasers. We propose that THG near the focal point of a tightly focused ultrashort pulse can be used as a microprobe for microscopical studies. Differences in THG coefficients at different locations in the sample translate to differences in THG intensities that can be easily measured. This principle can therefore be used to form a nonlinear laser scanning microscope. We note that second harmonic generation (SHG) was used in the past for laser scanning microscopy,[1,2] however, unlike THG, it is limited to the observation of noncentrosymmetric media such as crystals or structured media.

Consider a laser beam propagating in the z direction and tightly focused into a sample. Using the theory of harmonic generation with focused Gaussian beams, we can calculate the intensity of the third harmonic light under the undepleted-pump approximation. The third harmonic power generated by such a beam is given by[3]

$$P_{3\omega}=k_{3\omega}k_{\omega}^{3}\left(\frac{4\pi}{n_{3\omega}n_{\omega}^{2}c}\right)^{2}P_{\omega}^{3}|J|^{2}, \tag{1}$$

with P_{ω} the fundamental beam power, k_{ω} and $k_{3\omega}$ are the fundamental and third harmonic wave numbers, and the integral J is defined by

$$J=\int_{z_L'}^{z_R'}\frac{\chi(z')e^{i\Delta kbz'}\,dz'}{(1+2iz')^{2}}, \tag{2}$$

where χ is the third-order susceptibility, $\Delta k=k_{3\omega}-3k_{\omega}$ is the phase mismatch, $b=k_{\omega}\omega_0^2$ is the confocal parameter of the fundamental beam with a waist radius of ω_0, $z'=z/b$ is the normalized coordinate along the optical axis measured from the beam waist position, and z_L' and z_R' are the normalized input and output planes coordinate, respectively. In the case of an infinite, uniform nonlinear medium, this integral can be evaluated by contour integration. The result of this integration is somewhat surprising: The efficiency of THG in this limit vanishes for the case of positive phase mismatch ($\Delta k>0$, which is the case of normally dispersive materials) and even for perfect phase matching ($\Delta k=0$). The need for negative phase mismatch can be understood if we remember that a focused beam contains a collection of wave vectors in many directions. Three such wave vectors can add to give $k_{3\omega}$ only when $k_{3\omega}\leqslant 3k_{\omega}$ (see Ref. 3).

When the nonlinear medium is not uniform, either in the refractive index or in the nonlinear susceptibility, the THG signal does not vanish, and significant THG output can be obtained. For example, near an interface between two media with the same linear refractive index but with different nonlinear susceptibility we calculate (assuming $\Delta kb\ll 1$) that $P_{3\omega}\propto\delta\chi^{2}(1+4z_{\omega}^{2}/b^{2})^{-1}$, where $\delta\chi$ is the difference in susceptibility values and z_{ω} is the distance between the interface and the beam waist. Hence, as this interface is scanned along the optical axis the THG signal is generated efficiently when the interface is near the beam waist. The signal peaks when the interface is at the beam waist position, and its full-width at half-maximum (FWHM) is b. When the linear index and the phase mismatch are also discontinuous at the interface, the beam parameters change across the interface, but the third harmonic is still generated efficiently only when the interface is located within one confocal parameter of the focal plane. Similarly, a thin film with a thickness $t\ll b$ embedded in a homogeneous medium will generate a THG signal with

$$P_{3\omega}\propto\delta\chi^{2}(t/b)^{2}(1+4z_{\omega}^{2}/b^{2})^{-2}. \tag{3}$$

Again we see that the signal is generated only when the film is near the focal plane, now with a FWHM of $0.64b$.

We have studied experimentally THG with a tightly focused beam and its axial resolving power. THG near interfaces was studied recently in gas cells[4] and in various solids,[5] studies that were motivated primarily by the quest for efficient THG sources. Here we examine the suitability of this technique as a nonlinear microprobe for studies of transparent objects. Our light source was a synchronously pumped optical parametric oscillator (OPO) with a 125 fs

[a]Electronic mail: feyaron@wis.weizmann.ac.il

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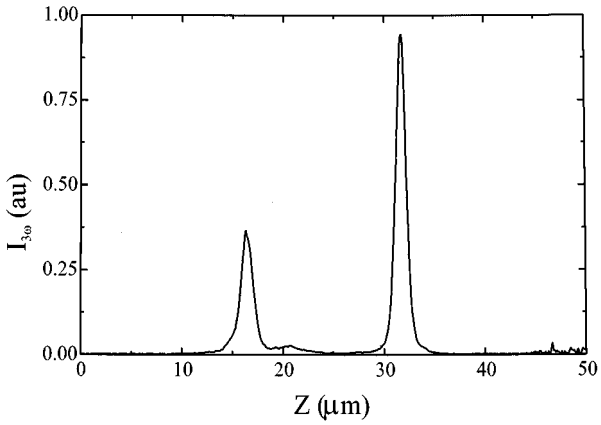

FIG. 1. Third harmonic power as a function of sample position along the focused laser beam. The sample is composed of a layer of index-matching oil between two glass plates separated by 15 μm. The beam is focused by a $\times 100$ objective. The two THG peaks correspond to the glass–oil interfaces crossing the focal point. THG is generated only within 1.2 μm of the interfaces.

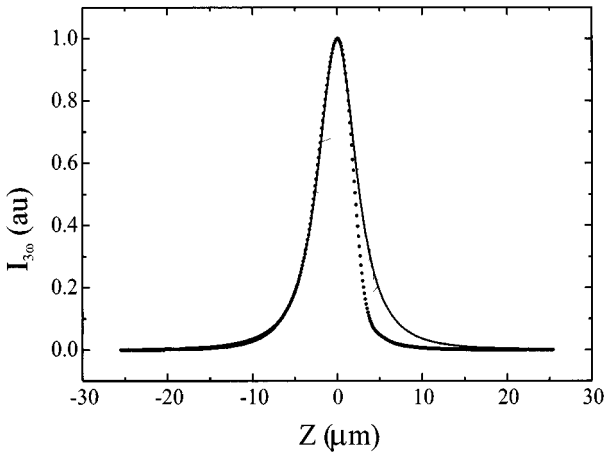

FIG. 2. THG at a air–glass interface as a function of the interface distance from the focal point. The objective is $\times 60$, NA 0.85. The circles are experimental measurements, the line is a theoretical fit.

pulse duration, 80 MHz repetition rate, and an average power of 380 mW, operating at a wavelength of 1.5 μm. The beam was focused using high-power microscope objectives, the fundamental wave was filtered out, and the third harmonic light intensity was measured using a photomultiplier and a lock-in amplifier. The light intensities used in these experiments were between 20 and 200 mW of average power, and the maximal THG signals were of the order of a few nW.

To measure the depth resolution of this technique, we used a sample made of a thin film of index-matching oil ($n=1.516$) between two glass plates separated by 15 μm spacers. The sample was scanned along the optical axis of a $\times 100$ objective (Zeiss Plan-Apochromat, NA 1.4). Figure 1 shows the third harmonic intensity as a function of axial position. The two peaks of third harmonic light seen in the figure correspond to a beam waist at the glass-to-oil and oil-to-glass interfaces in the sample. The FWHM of both peaks are approximately 1.2 μm and the measured spacing between them is 15.4 μm. This width is consistent with the above theoretical analysis if the beam waist at the focal point is approximately $0.5\lambda/n=0.5$ μm. Note that even though the linear index of the film is matched to the glass, the nonlinear susceptibility is different and this leads to a large signal at the interface.

We also scanned a single glass plate in the focal point of a $\times 60$, 0.85 NA lens. Figure 2 shows the observed THG signal at the air–glass interface (circles), which is asymmetric with respect to the focal point position. The growth of the signal as the beam waist approaches the glass matches well to a theoretical fit (solid line in Fig. 2) with a beam waist of $0.8\lambda/n=0.8$ μm and a linear index mismatch of $n_{3\omega}-n_{\omega}=0.025$. Since the theory predicts a symmetric response for such an interface, we believe that the asymmetry is caused by aberrations of the Gaussian beam as it crosses from air into the glass.

We also performed two-dimensional scans of a sample of glass fibers mounted between two glass plates and immersed in index-matching fluid. The sample is scanned in steps of 5 μm along the axial and one transverse directions. The resulting image is shown in Fig. 3 where the THG signal intensity is coded as gray levels, displayed in a logarithmic scale. The interface between the fibers and the oil is easily resolved, as well as a feature at the fiber core. We also observe a distortion of the image in the depth direction, as well as a decrease of THG efficiency with depth, which again we attribute to the aberration in the lens. Scanning of the sample in this work was accomplished using stepper-motor driven stages, and these limited the speed of the scans to about 20 s per line. We estimate that by scanning the optical beams, as in a conventional scanning laser microscope, could reduce the scan time considerably.

Tsang recently reported[5] a study of THG at interfaces. He had attributed most of the THG signal to a surface effect, much like surface SHG which is generated by one or few atomic monolayers near the interface.[6] However, our results are consistent with the conventional theory of bulk THG near the interface as explained above. To verify this we have also checked the backward propagating THG and found it to be

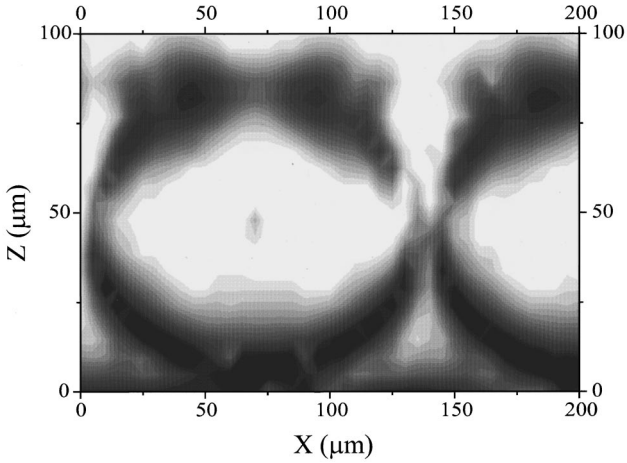

FIG. 3. THG mapping of a sample of optical glass fibers in index-matching oil. A cross-sectional view along the z (depth) and x coordinate is shown. The image was taken with 5 μm steps in both directions. Gray levels represent the intensity of the THG signal in a logarithmic scale. Strong THG signal is generated at the fiber–oil interface, and also near the core of the fiber.

negligible compared with the strong forward propagating signal. THG generated by a few atomic monolayers would radiate equally in the forward and backward directions.

Nonlinear techniques have been used in recent years in scanning laser microscopy; two-photon flourescence,[7] and more recently three-photon flourescence,[8,9] have been shown to increase the resolution and contrast in confocal microscopy as compared with single-photon flourescence techniques. In this letter, however, we have demonstrated a technique that is suitable for general transparent samples. The applicability of this technique to the observation of various type of samples is currently being investigated.

In conclusion, we have seen that THG generated by a tightly focused ultrashort pulse can be used to map the distribution of third-order susceptibility in transparent samples. The THG detects interfaces and inhomogeneities, and in that it resembles linear optics, where signals—reflections or scattering—are generated only at interfaces and inhomogeneities of the refractive index. All material have some nonvanishing third-order susceptibility; a simplified theory for dilute gases predicts that $\chi \propto n^8$. While solid media are not expected to follow this dependance, it is known that their nonlinear susceptibility does vary over many orders of magnitude, compared with linear index changes that vary in a relatively small range. Therefore, we expect that THG should be an efficient tool for mapping material distributions. With the advances in ultrashort pulse lasers there are several sources that produce 100 fs pulses with 10^3-10^4 W of peak power, or $10^{11}-10^{12}$ W/cm^2 at the focal point of a lens. Most suitable are laser sources in the $1-2$ μm wavelength range, where many samples of interest will be transparent for both fundamental and third harmonic light.

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