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Frequency Doubling and Tripling of Ultrashort Laser Pulses in Biological Tissues

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Abstract Structural proteins such as collagen and elastin are known to generate second harmonic at high laser intensities. Second and third harmonic generations (SHG, THG) of 0.4 ps Ti-Sapphire laser radiation at 800 nm were observed in various biological tissues. Dependence of SHG on laser pulse energy and pulse width was investigated. Reflected second harmonic yield was measured for animal tissue *in vitro* and human skin *in vivo*. The yield varies about a factor of 20 for various areas of the skin while the scattered laser radiation (diffuse reflectance) varies only by a factor of 2. In some cases the THG efficiency was comparable to the SHG. Possible applications of higher harmonic radiation for diagnostics and microscopy are discussed.

1. Introduction

It is well known that biomolecules have nonlinear optical properties such as frequency mixing, higher harmonic generation, and phase conjugation at short laser pulses with high energy per pulse [1]-[4]. However, the nonlinear optical properties of biological tissues irradiated by ultrashort laser pulses are widely unknown. Pioneering work was performed by Hovannessian and Lalayan using 40 ps Nd:YAG laser at 1064 nm [5]. They observed second harmonic generation (SHG) in various animal tissues and attributed the effect to the presence of fibril proteins. The effect was not found in plant tissue. Later, based on a paper on intense third harmonic generation at interfaces of crystals and other solids [6], Squier et al. studied third harmonic generation (THG) in biological material [7]. Using 100 fs laser pulses with a wavelength of 1.2 μm they measured third harmonic signals in plant leaves.

The study of SHG and THG in biological tissue is of significant interest due to the following reasons: (1) It was shown that third harmonic laser microscopy is possible, yielding new information about materials [7],[8]. Also, due to strong second harmonic yield in fibril containing tissue, scanning microscopy can be developed; (2) Harmonic wavelength could be used for tissue characterization during ablation [9]; (3) Since harmonic generation is a nondestructive method, it can be an important optical biopsy technique.

2. Experiments

The laser system is equipped with a Ti-Sapphire oscillator and a regenerative amplifier. The oscillator is actively mode-locked at 82 MHz (Spectra Physics, 3960) and pumped by a 5 W frequency doubled Nd:YAG laser (Spectra Physics, Millenia) running at 532 nm. The oscillator pulse has a duration of 80 fs (FWHM) at 800 nm. Its pulses are amplified by a Ti-Sapphire regenerative amplifier (Positive Light, Spitfire) through a chirped pulse amplification process. This amplifier is pumped by a 10 W, 527 nm Nd:YLF laser (Positive Light, Merlin). The maximum pulse energy is about 1 mJ with variable pulse duration of 0.15 ps - 20 ps.

The 400 fs pulse train was used to irradiate various parts of pork and chicken tissues *in vitro* and human skin *in vivo*. The beam diameter was approximately 130 μm and the beam intensity was much smaller than the tissue damage threshold. The reflected second and third harmonics were collected by 1 mm diameter optical fibers and analyzed using a spectrometer and photomultiplier setup. The spectrometer was calibrated using a mercury lamp and a narrow slit was used to detect only the 400 nm doubled or 266 nm tripled light. We directly illuminated the 1 mm detector fiber using the source laser to confirm that no doubled or tripled light was generated in the optical system.

3. Results and Discussions

Higher harmonic generation was observed in various kinds of tissues including pig, chicken, and human skin. Fig. 1 shows that the SHG intensity increases quadratically with the incident laser power, as it is expected for a second order process. However, higher order processes for frequency doubling are also possible [2]. The first harmonic represented by the scattered laser radiation increased linearly with increased intensity.

The yield of second harmonic radiation was measured in teeth for a pulse width from 0.4 to 10 ps (Fig. 2). At constant pulse energy, the second harmonic signal is expected to be inversely proportional to pulse width. However, the signal decays slower than expected, which could be due to pulse shape changes which need to be quantified.

Table 1 shows the relative intensity of second harmonic radiation generated in various tissues. A significant variation in doubled light intensity was observed for different regions of various samples. The intensity varies about a factor of 20 at different areas of the human skin *in vivo* while the scattered light (first harmonic) shows variations of only a factor of 2. Similar results were obtained in tissues of pig, chicken, and extracted human tooth *in vitro*. The results are in agreement with the general tendency found by Hovanessian et al. [5], indicating a correlation between the yield of second harmonic generation and the content of fibril proteins, especially collagen.

The effect of the incident beam polarization on the second harmonic yield in tendon and the outer part of the vertebra was investigated. The second harmonic generation varies about a factor of 2 for tendon and 1.5 for vertebra with polarization changes, while the scattered laser radiation remains constant.

The third harmonic radiation was detected from teeth. Even if our detector system was not very effective at 266 nm region, we observed significant THG which is comparable to SHG. It is still not clear if the third harmonic signal is generated from tissue surface or from the bulk material. Further investigation will be conducted and reported in the near future.

4. Conclusions

It was demonstrated that relatively strong second and third harmonic radiation in tissue occurred using 0.4 to 10 ps laser pulses. Further studies are required to investigate if this type of radiation can be applied in laser surgery as a tool for diagnostics of selective tissue ablation or in microscopy as mentioned in the introduction.

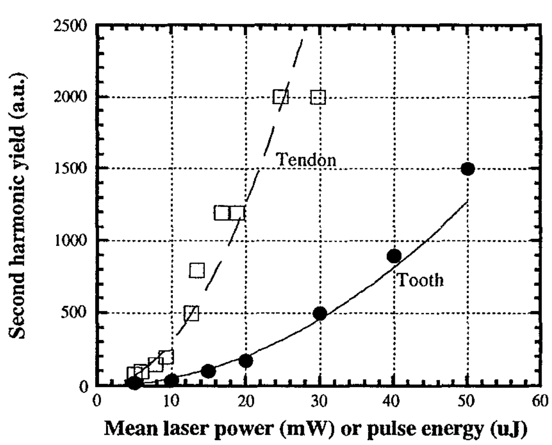

Fig. 1: Dependence of second harmonic radiation on laser power for human tooth and chicken tendon (Ti-sapphire laser 800 nm, 0.4 ps, 1 kHz, beam diameter = 0.113 mm).

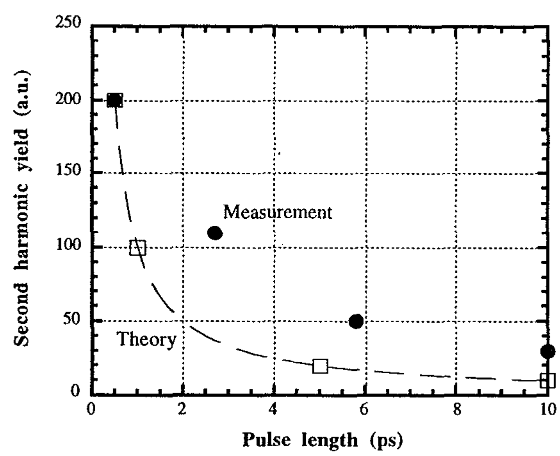

Fig. 2: Relative intensity of the second harmonic generation with various pulse widths (Ti-sapphire laser 800 nm, 0.4 ps, 1 kHz, beam diameter = 0.113 mm, laser power 50 mW)

Table 1: Relative intensity of 2nd harmonic and scattered laser radiation in animal tissue (Ti-sapphire laser 800 nm, beam diameter = 1 mm, 0.4 ps, 1 kHz, pulse energy 0.1 mJ)

Tissue	Part	Intensity of Second Harmonic [a.u.]	Intensity of Scattered Light [a.u.]
Human hand and arm (*in vivo*)	Fingernail	5	210
	Finger tip	10	140
	Finger skin upside	50	150
	Hand lentigo (back side)	50	150
	Hand near lentigo (back side)	100	150
	Palm (middle inner part)	100	220
	Wrist near pulse	170	290
Pig (*in vitro*)	Muscle (near vertebra)	10	220
	Muscle with small tendon	70	140
	Rib section (inner part)	20	100
	Rib section (outer part)	80	160
	Vertebra section (inner part)	40	150
	Vertebra section (outer part)	190	110
	Tendon	700	130
Chicken (*in vitro*)	Muscle	1	500
	Fat	1	400
	Skin	600	230
	Cartilage	650	260
	Tendon	1600	400
Tooth (*in vitro*)	Human Tooth	300	400

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