Dependence of second-harmonic signals generated in malignant human prostate tissue on excitation wavelength

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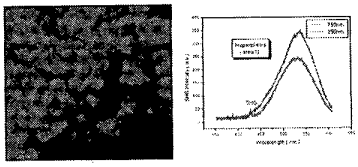
Abstract: Second-harmonic signals were measured from hyperplastic parenchyma and stroma in malignant human prostate tissue under femtosecond pulsed illumination at different excitation wavelengths. The dependence of the second-harmonic generation on the excitation wavelength provides a possible indicator for recognising tissue components and malignancy.

Second-harmonic signal results from the second-order nonlinear optical susceptibility. Such a physical property is determined by the electronic configuration, molecular symmetry, local morphology, orientation, and alignment of molecules and ultrastructures. Second-harmonic generation (SHG) in biological tissue was first reported by Fine and Zaret[1, 2] in 1965. Fine and Hansen reported the SHG produced in corneas, scleras, tendon, skin of dog and rabbit at an excitation wavelength of 694 nm.[3] Recently, SHG was measured in chicken muscle and skin at an excitation wavelength of 810 nm. The fact that SHG shows the strong structural dependence[4-6] in biological tissue may offer a potential, as a noninvasive tool, for exploring tissue components, tissue environments and further disease diagnosis.

We have studied SHG in malignant human prostate tissue in order to use it as a signal for distinguishing normal and abnormal tissue structures. In particular the dependence of SHG from hyperplastic parenchyma and stroma in malignant human prostate tissue on the excitation wavelength has been measured.

The experimental setup included a femtosecond pulsed laser (Spectra-Physics: MaiTai) of a pulse width between 70~100 fs and a wavelength-tuning range from 730 ~ 870 nm, a scanning microscope (Olympus: FluoView300), which provided three-dimensional imaging, and a spectrograph (Acton Research Corporation: SpectroPro-300i). Samples from human prostate tissue were freshly frozen. All spectra were measured under the given excitation power of 10 mW at excitation wavelengths of 730 nm, 750 nm, 800 nm, 850 nm and 870 nm, respectively.

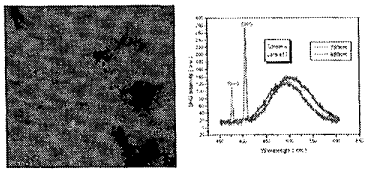


(a) (b)

Figure 1 SHG in hyperplasia: (a) image; (b) spectra at two excitation wavelengths.

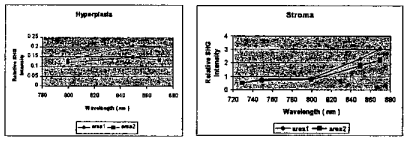
Fig. 1(a) shows the image of hyperplasia while Fig. 1(b) shows the spectra in a given area (area1) at excitation wavelengths 750 nm and 850 nm, respectively. The sharp peaks in Fig. 1(b) represent the SHG spectra while the broad peaks correspond to two-photon fluorescence spectra. Figs. 2(a) and 2(b) show the corresponding results from stroma. Fig. 3 displays the relative intensity of SHG as a function of the excitation wavelength in hyperplasia (a) and in stroma (b), respectively. The relative intensity refers to the ratio of the

intensity to the two-photon fluorescence intensity under the same excitation condition.



(a) (b)

Figure 2 SHG in stroma: (a) image; (b) spectra at two excitation wavelengths.



(a) (b)

Figure 3 Dependence of SHG on the excitation wavelength in hyperplasia (a) and stroma (b).

In hyperplasia, the strength of the SHG signal can be negligible for the excitation wavelength shorter than 750 nm. We can also see from Fig. 3(a) that SHG is the strongest at excitation wavelength 850 nm. Such a maximum does not occurs in stroma. In comparison with stroma, the intensity of the SHG in hyperplasia is about 10 times weaker. From these results, we can conclude that SHG in prostate tissue is highly structure and wavelength dependent, which may be used an indicator for recognising tissue components, ultrastructures, and micro-environments, and further for disease diagnosis.

Because of the complex of biological tissue, we can focus only on the main factor which may cause the difference of the strength in SHG in different kinds of tissue. The experimental results by other researchers[5] showed that the magnitude of the SHG signal is strongly structure dependent; the signal from chicken skin is the strongest, the signal from muscle is the second strongest, and the signal from fat is the weakest. Our other experimental results (not shown here) on the muscle of Balb/ce mice also exhibited strong SHG. Considering the histologic structures of these tissues, we can see that skin includes lots of collagenous fibres and that skeletal muscle and stroma are composed of collagen fibres[7-9]. However in fat and hyperplasia, there are few collagen fibers.[7-9] This analysis suggests that collagen fibres may be one of the main sources which causes strong SHG.

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