Nonlinear photoacoustic generation by pump-probe excitation

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ABSTRACT

Photoacoustic technique has undergone major developments in recent decades. Most of current photoacoustic systems work in the linear range, in which the photoacoustic amplitude increases proportionally to the increasing of illuminating light power. The imaging sensitivity and contrast, however, in this case are limited due to stronger background signals from intrinsic chromophores in biological tissue. The current work investigates the advantages of nonlinear photoacoustic generation compared to linear signal, by using a single-wavelength pump-probe and nanosecond-scale two-pulse excitation scheme. The results show that nonlinearity induced by this scheme yields higher detection sensitivity and contrast.

Keywords: photoacoustic, nonlinearity, pump-probe

1. INTRODUCTION

Pump-probe microscopy is a kind of microscopies that uses a pump beam and a probe beam combined together with certain time delays between them, to get improved molecular specificity, penetration depth and resolution[1.] Multiple parameters, such as wavelength, power, polarization, modulation frequency etc, of each beam can be adjusted to fulfill a specific detection objective. The big available parameter space has made pump-probe microscopy a powerful tool that has great applications such as extracting specific structural features from dynamical phenomena[2], and differentiating molecules with extremely similar linear spectrum.

Different from conventional fluorescence microscopes that only fluorescent molecules can be imaged, pump-probe can give epidetection of fluorescent[3] as well as non-fluorescent molecular contrast by detecting the modulated pump induced changes in the transparent or backscattered probe beam. This has greatly expanded contrast sources of molecular imaging. As in many cases, there are not many endogenous fluorescent molecules in vivo; for the those having fluorescence, their super broad excitation and emission spectrum make it difficult to get reliable information from the fluorescent signals. However, for epi-fluorescence detection, only very thin sample (<1mm) is suitable; for transparence detection, only transparent or sample with little scattering is suitable. For thick tissues, backscattering probe photons that carry the modulation frequency of the pump beam are detected, in this case, the efficiency is not high enough with only one in a million of pump modulation may be transferred to probe beam, and thus careful stripping of pump beam is essential, which is quite difficult; the resolution in backscattering mode also cannot be protected inherently.

Photoacoustic (PA) imaging, combining light absorption with acoustic detection, has gained significant development in recent decades. Different from photomultiplier or photodiodes detectors that have no focusing ability and often used in conventional pump-probe microscope setups, acoustic resolution can be retained in PA imaging with much deeper penetration depth by using a focused ultrasound transducer to detect photoacoustic signal that excited by one-way transmitted photons with red wavelengths within the second biological window (700-1100 nm)[4-6]. Recently, researchers have tried to combine pump-probe excitation with photoacoustic detection. For example, stimulated Raman for molecular specific photoacoustic imaging[7], enhanced PA signal based on stimulated emission[8-9], two-photon absorption photoacoustic microscopy with enhanced z-axis resolution[10], molecule differentiation[11-13], etc.

In most of these studies, however, two short-pulsed multi-wavelength laser sources are needed for pump and probe, respectively, to fulfill applications for different molecules. This is convenient but has dramatically increased the cost and complexity of the whole system. As we can see, “Pump-probe microscope” is a very general expression which includes a bunch of microscopes based on different nonlinear optical processes, such as harmonic generations, stimulated Raman scatterings, and transient absorption processes, for details we refer readers to a review article[1]. Except from a few

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examples mentioned, most current photoacoustic technique work with linear absorption, and the huge advantages that provided by nonlinear optical response remain to be explored, especially when combined to a specific nonlinear optical configuration. In the current study, we built a pump-probe photoacoustic (PP-PA) system and demonstrated the nonlinear property of the signal and its higher sensitivity.

2. METHODS

The experimental setup is shown in Figure 1. An Optical Parametric Oscillator (OPO) with 100 Hz repetition rate and ~5 ns pulse width was used as the light source. Light illuminating from the laser source was firstly collimated and expanded, and the high-frequency components were filtered by a pinhole at the first focal point. An iris was placed after the expanded beam to adjust the beam width to match the back aperture of the objective. The beam was then split into two beams by a half-wave plate and a polarized beam splitter. The probe beam can be continuously delayed by using a motor rail and reflection mirrors in nanoseconds and then reflected back and combined again with the pump beam by a second beam splitter. The pump and probe powers were easily tuned by the half-wave plate and the polarized beam splitter. A photodiode was placed after the second beam splitter to monitor a sampled portion of the exciting light for normalization of signal fluctuation caused by the unstable power of laser pulses. A focused ultrasound transducer was placed in transmission mode and the acoustic focal area was carefully adjusted by 3D manual translation stages to match the light focus to get highest detection sensitivity. In this study, we chose the commonly used water solution of methylene blue (MB) molecules as the testing sample.

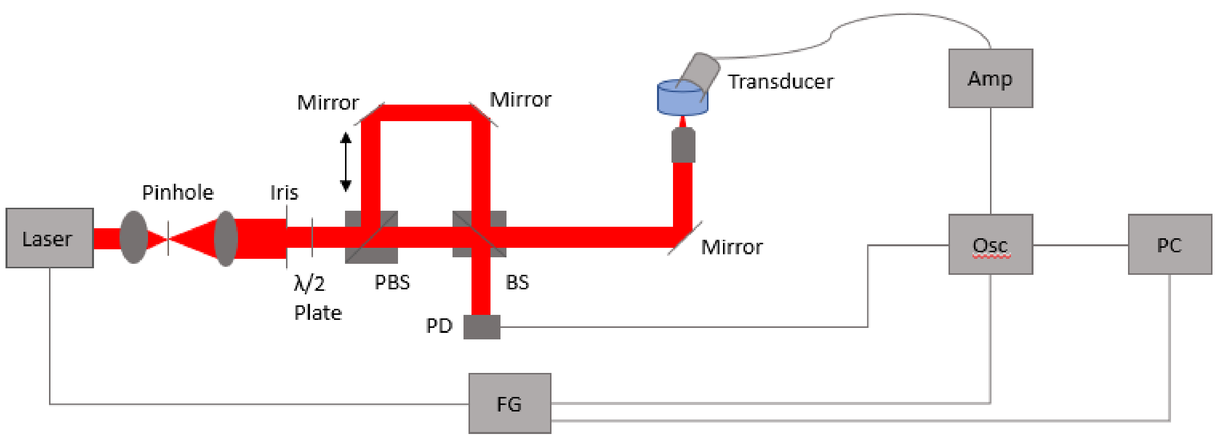

Figure 1. Pump-probe photoacoustic excitation scheme. PBS: polarized beam spliter; BS: beam spliter; PD: photodiode; FG: function generator; Amp: amplifier; Osc: oscilloscope; PC: personal computer.

3. RESULTS AND DISCUSSIONS

To make sure that response to single laser pulse is a linear process, we first tested the realtionship between laser power and PA amplitude by using only the pump pulse. As shown in Figure 2, PA signal amplitude increases linearly with increased laser power till 3 mW single-pulse excitation. After 3mW, cavitation and PA signal of the water background appear with the high NA objective used in this setup, which are nonlinear phenomenon and were thus excluded.

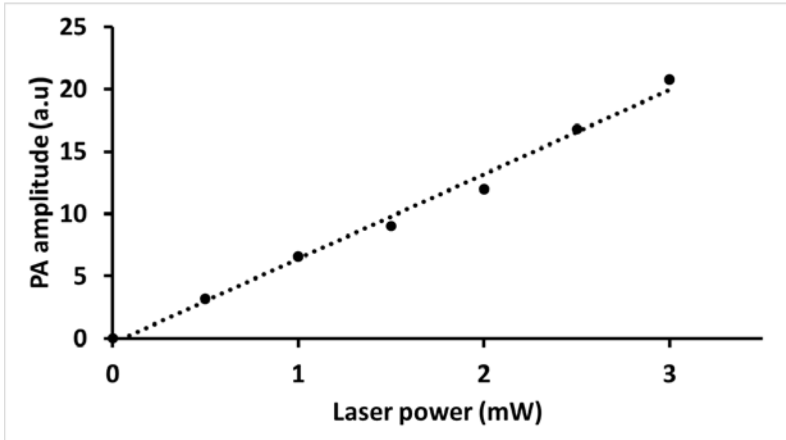

Figure 2. Linear power range of single-pulse excitation response.

Figure 3 shows the A-line signals with three schemes of excitation, respectively. With probe beam only, there's no detectable signal (black); with the pump beam only, the signal increased a little bit (red); with combined pump-probe excitation, the signal increased dramatically (blue). Compared to the 1.5 times of excitation power increasing from 2mW to 3mW, the peak-to-peak PA amplitude has more than 3 tmes of increasing from 20 (a.u) to 65 (a.u), which is a strong sign of nonlinearity. This shows that nonlinear response was realised in linear-response power range by using pump-probe excitation scheme.

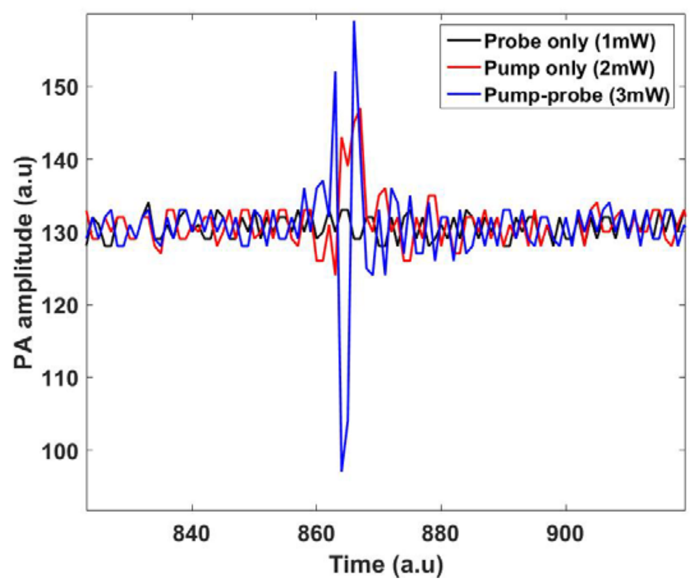

Figure 3. Photoacoustic signals got from different excitation scheme.

We further investigate the effects of excitation power of the two pulses and delay time of the probe beam on the signal amplitude. As shown in Figure 4, the probe beam was delayed 1.5 ns and 3.7 ns, respectively. Under each delay time, the ratio between pump and probe beam power was adjusted among three levels, 0.5, 1, and 2 times. We found that when pump power is two times of probe power, and when the probe beam has shorter delay, 1.5ns in this case, the signal can be most amplified. While when the pump power is half of the probe power, and when the probe beam has longer delay, 3.7 ns in this case, the singal was least amplified. This result shows that the higher pump power, the higher nonlinear efficiency, and the shorter probe delay time, the higher nonlinear efficiency.

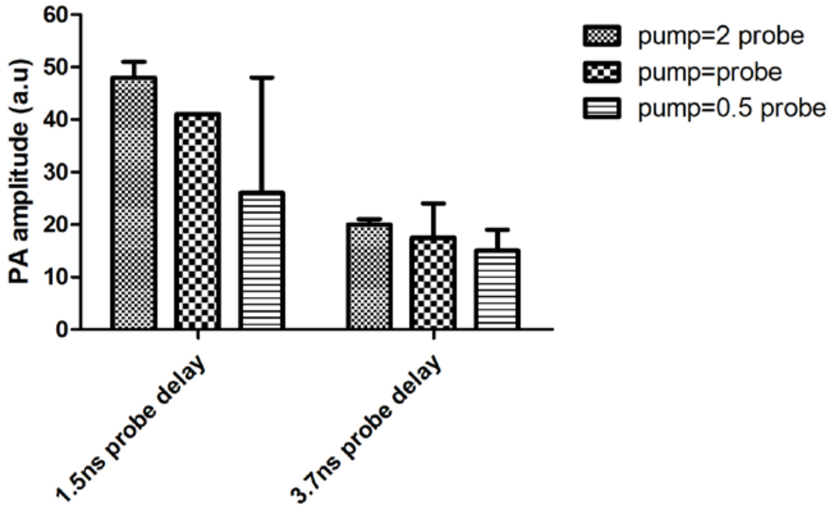

Figure 4. Effects of probe delay time and ratio of pump-to-probe power on nonlinearity.

Knowing the most efficient way to get nonlinear signal amplification, we next investigated the detecting sensitivity that can be realized under this scheme. As shown in Figure 5, by one-pulse stimulation (probe only), there is no detectable difference between 2 μM and 1 μM or background. While by pump-probe stimulation, there shows a 35% higher signal amplitude of 2 μM solution compared to 1 μM solution, which is enough to be differentiated. As normally an increase between 5%-10% compared to noises can be regarded as detectable signal, the detection limit can be less than 2 μM in more detailed experiments.

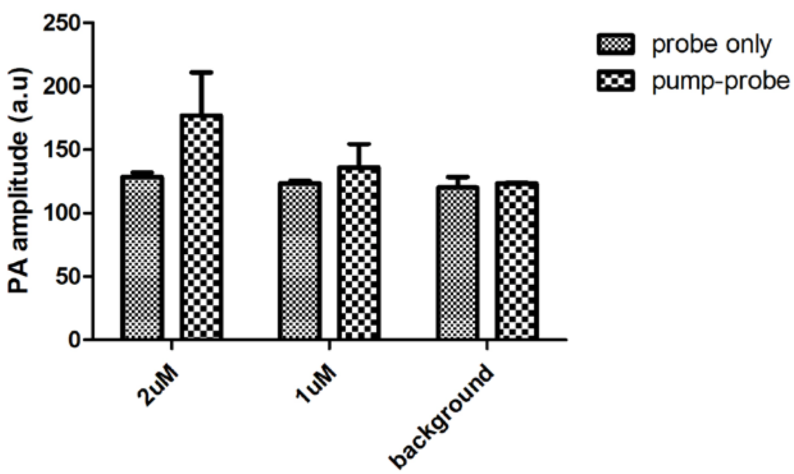

Figure 5. Pump-probe excitation provides higher sensitivity.

4. CONCLUSION

In this study, we investigated photoacoustic nonlinearity by using a pump-probe excitation method, demonstrating that nonlinearity can be excited in linear power range by using this scheme, and that higher detection sensitivity can be realized. Understanding of nonlinear photoacoustic mechanisms and applications based on these finds will be further studied to show the advantages of nonlinear photoacoustic imaging that have not yet been fully explored.

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