Nonlinear Photoacoustic Imaging by Pump-Probe Excitation

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Abstract: Nonlinear photoacoustic imaging is demonstrated by a pump-probe photoacoustic excitation scheme by using PPIX. The system has potentials to greatly facilitate deep-tissue photoacoustic theranostics as well as high resolution dynamic photoacoustic molecular imaging.

OCIS codes: (110.0110) Imaging systems; (110.5120) Photoacoustic imaging

1. Introduction

Molecular imaging enables the visualization, characterization, and quantification of biologic processes taking place at the cellular and subcellular levels within intact living subjects including patients [1]. Studying these kinds of processes, especially cellular and molecular pathways, or mechanisms of disease in their physiologically authentic environment instead of *in vitro* or *ex vivo* is greatly pursed. For dynamic molecular imaging, one of the methods is using pump-probe microscopy, an emerging tool with superior detection sensitivity, chemical specificity and spatial-temporal resolution that can reveal intrinsic molecular dynamics [2]. Conventional pump-probe systems measure the change in the transmission of a probe beam induced by a pump beam, which limits the *in vivo* applications of these systems. Photoacoustic (PA) imaging, which combines sensing of optical contrast with acoustic resolution in deep tissue, has attracted tremendous attention in recent years [3]. PA has ushered in a new era of biomedical imaging and has facilitated the investigation of fundamental biological mechanisms and clinical translational applications [4]. By translating transmitted optical intensity detection to all-around acoustic detection, we here developed a pump-probe photoacoustic microscopy which can yield *in vivo* molecular dynamic imaging. PPIX, a commonly used photosensitizer, which can also be induced inside cells by treating cells with drugs, which has one-photon peak absorption at 405 nm is used in this study to demonstrate the nonlinear performance of the PA system.

2. Results and discussion

The setup is shown in Fig. 1. An OPO (Optical Parametric Oscillator) was utilized as the excitation source. Its output was split by a first beam splitter into one pump beam and one probe beam, which were recombined again with a second beam splitter. The recombined beam was then shaped and focused onto the experiment sample. A 10-

MHz transducer was positioned in transmission mode to receive the generated PA signals. A photodiode was used to monitor the fluctuations of the laser output power.

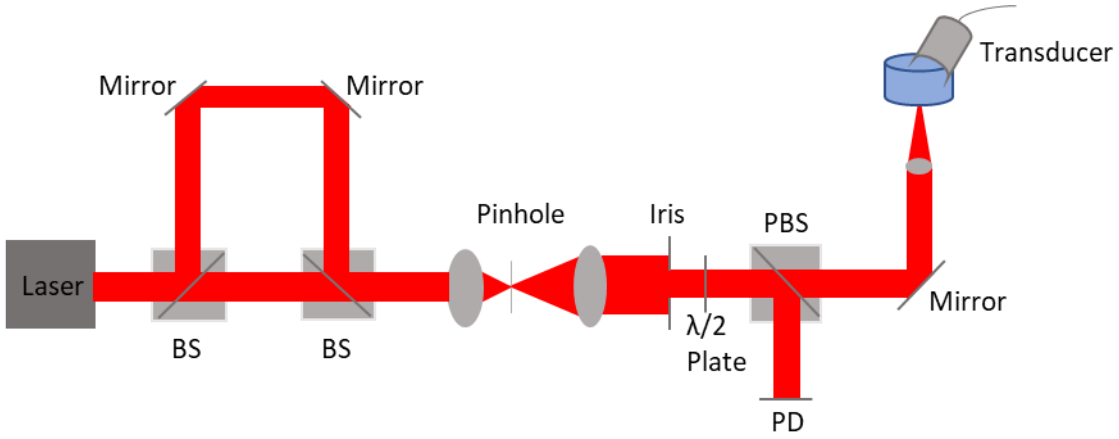

Fig. 1. Scheme of the pump-probe PA microscope

PPIX was dissolved in DMSO (dimethylsulfoxide). Fig. 2. (left) shows the absorption spectral recorded by a UV-VIS microplate reader. A strong absorption peak is located at about 405 nm, and nearly no absorption can be seen beyond 700 nm. The photoacoustic spectral is shown in Fig. 2. (right). The signal amplitude under each wavelength was normalized to its corresponding laser power indicated by the photodiode reading to remove the power fluctuation effects. A main peak at 750 nm and a subsidiary maximum appears at 820 nm, which is near the twice of the peak of PPIX at 405 nm. According to the nonlinear theory, this phenomenon can be explained by the nonlinear absorption of the low-frequency photons.

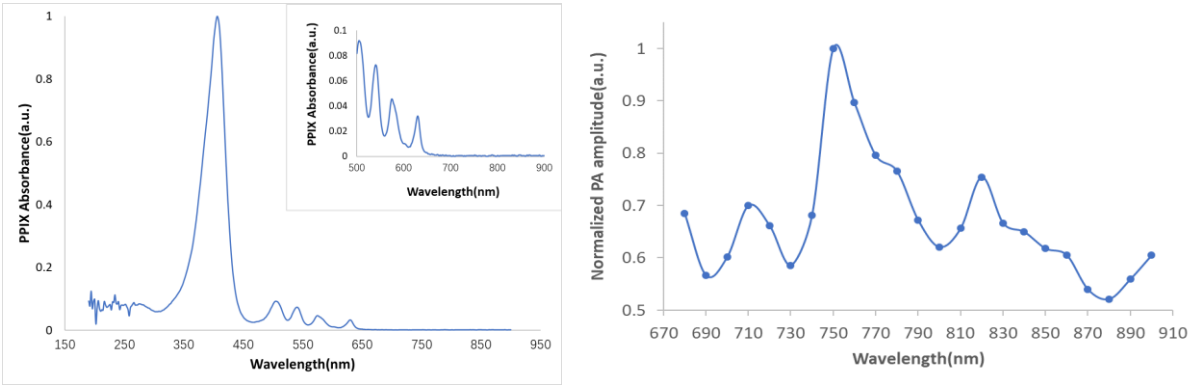

Fig. 2. Absorption spectral of PPIX (left) and the photoacoustic spectral in the near-infrared region (right).

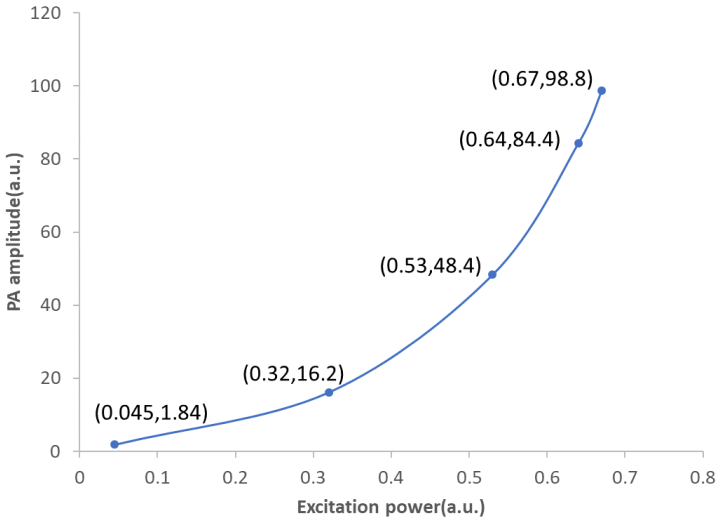

Fig. 3. PA amplitude versus excitation power

To confirm this nonlinearly, the photoacoustic amplitude was investigated as a function of the excitation power. The result is shown in Fig. 3. As seen that within the low power range (<0.32 a.u.), the PA amplitude increases approximately linearly with the excitation power. Beyond the low power range, PA signal amplitude increases more rapidly and is nearly proportional to the square of the excitation power increment.

3. Conclusion

A pump-probe photoacoustic microscopy with multi-wavelength laser source is set up, and a kind of porphyrin, PPIX, is used to demonstrate nonlinear photoacoustic performance. The feasibility of using this contrast agent for *in vivo* photoacoustic molecular dynamic imaging is demonstrated.

4. References

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