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Wavelength-Modulated Differential Photoacoustic Spectroscopy (WM-DPAS): Theory of a High-Sensitivity Methodology for the Detection of Early-Stage Tumors in Tissues

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Abstract In the field of medical diagnostics, biomedical photoacoustics (PA) is a non-invasive hybrid optical-ultrasonic imaging modality. Due to the unique hybrid capability of optical and acoustic imaging, PA imaging has risen to the frontiers of medical diagnostic procedures such as human breast cancer detection. While conventional PA imaging has been mainly carried out by a high-power pulsed laser, an alternative technology, the frequency domain biophotoacoustic radar (FD-PAR) is under intensive development. It utilizes a continuous wave optical source with the laser intensity modulated by a frequency-swept waveform for acoustic wave generation. The small amplitude of the generated acoustic wave is significantly compensated by increased signal-to-noise ratio (several orders of magnitude) using matched-filter and pulse compression correlation processing in a manner similar to radar systems. The current study introduces the theory of a novel FD-PAR modality for ultra-sensitive characterization of functional information for breast cancer imaging. The newly developed theory of wavelength-modulated differential PA spectroscopy (WM-DPAS) detection has been introduced to address angiogenesis and hypoxia monitoring, two well-known benchmarks of breast tumor formation. Based on the WM-DPAS theory, this modality efficiently suppresses background absorptions and is expected to detect very small changes in total hemoglobin concentration and oxygenation levels, thereby identifying

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pre-malignant tumors before they are anatomically apparent. An experimental system design for the WM-DPAS is presented and preliminary single-ended laser experimental results were obtained and compared to a limiting case of the developed theoretical formalism.

Keywords Biomedical imaging · Breast cancer · Differential photoacoustics · Wavelength modulation

1 Introduction

Biomedical photoacoustic (PA) imaging has a unique hybrid capability based on optical-to-thermoelastic energy conversion which provides the methodology with both the high contrast of optical imaging and the high resolution of ultrasound at the same time. In the Center for Advanced Diffusion Wave Technologies group (CADIFT), a new PA imaging modality, frequency domain photoacoustic radar (FD-PAR), has been developed based on frequency-modulated (chirp) optical excitation and frequency domain signal processing methods. FD-PAR has successfully produced images at ∼20 mm depth with sub-mm spatial resolution that are comparable to those generated by pulsed photoacoustic and ultrasound imaging [1,2]. The current study introduces a newly developed theory about another FD-PAR technique, wavelength-modulated differential PA spectroscopy (WM-DPAS) detection methodology. The WM-DPAS system is expected to detect small changes in local total hemoglobin concentration and oxygen saturation levels which would identify pre-malignant tumors before they are detectable by other screening methods. In this paper, preliminary results of the WM-DPAS measurements were also obtained using a single-ended optical source and compared to the developed theoretical formalism.

2 WM-DPAS Theory

The WM-DPAS theory assumes two lasers of different wavelengths ($\lambda_A = 680\,\text{nm}$ and $\lambda_B = 808\,\text{nm}$) where each waveform is square-wave modulated and the two waveforms are at around 180° phase difference. Two specific wavelengths have been chosen based on their blood optical absorption spectrum that, at 680 nm, both molar extinction coefficients of oxy- and deoxy-hemoglobin show a noticeable difference while they overlap at 808 nm (isosbestic point). Since the PA signal is much more sensitive to changes in blood optical parameters at 680 nm, differential PA signals from those two wavelengths would allow one to detect very small changes in carcinogenesis benchmarks. Moreover, a differential method would suppress existing system noise or any other background absorption, contributing to the high sensitivity of the probe. The theoretical development of the WM-DPAS for blood oxygenation and deoxygenation sensing follows.

The hemoglobin absorption coefficient μ_a is a function of wavelength and of oxy- and deoxy-hemoglobin concentration C_ox and C_de. It can be described as

$$\mu_\text{a}(\lambda, C_\text{ox}, C_\text{de}) = \ln(10)e_\text{ox}(\lambda)C_\text{ox} + \ln(10)e_\text{ox}(\lambda)C_\text{de}, \tag{1}$$

where e_{ox} and e_{de} are extinction coefficients of oxy- and deoxy-hemoglobin at wavelength λ. Since total hemoglobin concentration (C_{Hb}) is the sum of oxy- and deoxy-hemoglobin concentrations, Eq. 1 can be rearranged,

$$\mu_a(\lambda, C_{Hb}, C_{ox}) = \ln(10)e_{de}(\lambda)C_{Hb} + \ln(10)[e_{ox}(\lambda) - e_{de}(\lambda)]C_{ox}. \quad (2)$$

Therefore, for the $\lambda_A = 680\,\text{nm}$ laser,

$$\mu_{aA}(C_{Hb}, C_{ox}) = \ln(10)e_{de}(\lambda_A)C_{Hb} + \ln(10)[e_{ox}(\lambda_A) - e_{de}(\lambda_A)]C_{ox}. \quad (3)$$

However, for the $\lambda_B = 808\,\text{nm}$ laser, the term $[e_{ox}(\lambda_B) - e_{de}(\lambda_B)]$ vanishes since it is at the isosbestic point. Therefore, the equation becomes

$$\mu_{aB}(C_{Hb}, C_{ox}) = \ln(10)e_{de}(\lambda_B)C_{Hb}. \quad (4)$$

Since the PA signal is proportional to the absorption coefficient of the sample, Eqs. 3 and 4 can be combined to represent the differential PA signal as

$$\begin{aligned} P_{AB} \propto (\mu_{aA} - k\mu_{aB}) &= \ln(10)\,[e_{de}(\lambda_A) - ke_{de}(\lambda_B)]\,C_{Hb} \\ &\quad + \ln(10)\,[e_{ox}(\lambda_A) - e_{de}(\lambda_A)]\,C_{ox}, \end{aligned} \quad (5)$$

where P_{AB} is a differential PA signal, μ_{aA} and μ_{aB} are the absorption coefficients of the absorber at specific wavelength A (680 nm) or B (808 nm), respectively, and k is a constant determined by the modulated amplitude ratio R (A_A/A_B) and phase difference $\mathrm{d}P(P_A - P_B)$ of the two lasers. From Eq. 5, the constant k can be adjusted so that the term $[e_{de}(\lambda_A) - ke_{de}(\lambda_B)]$ becomes 0. In this way, the differential PA signal will be highly sensitive to the optical parameter changes of blood, being solely dependent on C_{ox}.

3 WM-DPAS Simulation

Using the foregoing theoretical formalism, a corresponding simulation has been carried out with values of the optical parameters taken from normal, pre-malignant, and malignant colon cancer tissue measurements [3]. In the simulation, 680 nm and 780 nm lasers were square-wave modulated at 3.5 MHz. Specifically the 780 nm laser was chosen for the simulation for its high market availability compared to the 808 nm laser. First, the total hemoglobin of varying concentration was simulated over increasing amplitude ratio R at a fixed 181° phase difference and the lock-in differential phase response was analyzed as shown in Fig. 1a. The system was then tuned at $R = 1.47$ where pre-malignant blood concentration exhibits the steepest slope. Once tuned, as shown in Fig. 1b, c, the differential phase can be represented as a function of total hemoglobin concentration and oxy-hemoglobin saturation level that are two main indicators for breast carcinogenesis [3]. With the steep part of a tuned region, this system would allow very sensitive differential phase measurements when the tissue health state becomes pre-malignant. Differential amplitude values are not used for the

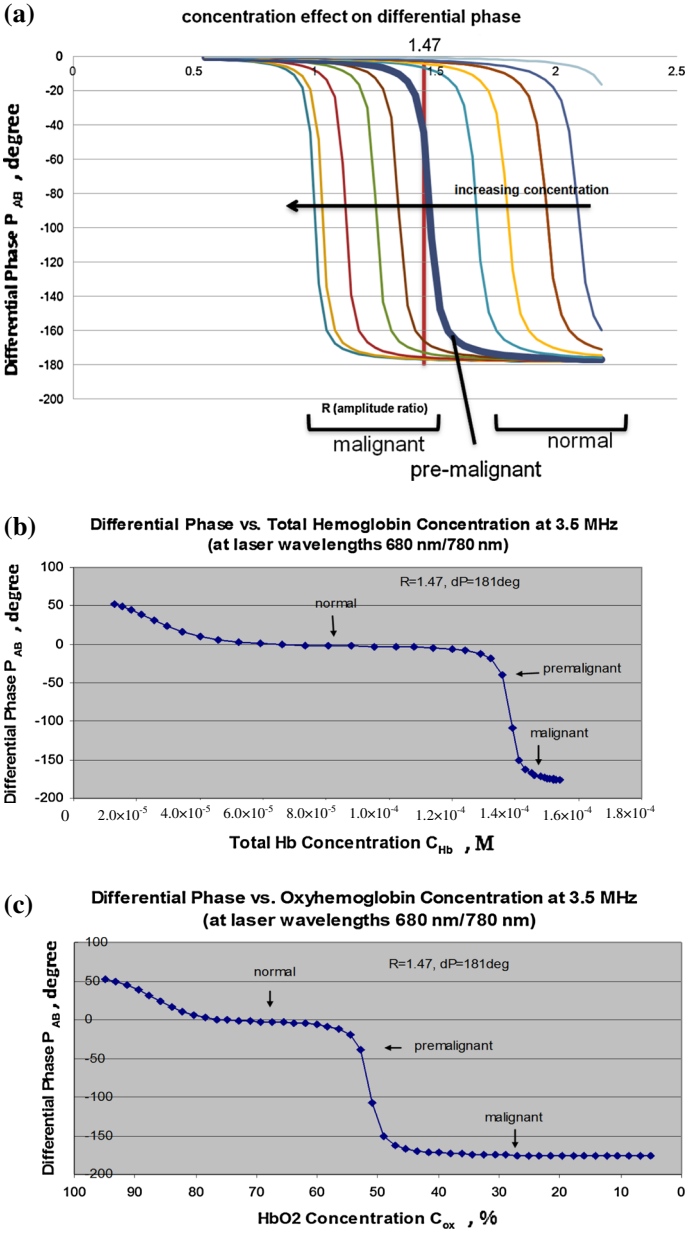

Fig. 1 (a) Differential phase simulation over increasing amplitude ratio R of two lasers (at fixed 181° phase difference). Four samples within the normal state range, one at the pre-malignant state, and five within the malignant state ranges were used to study the hemoglobin concentration effect on differential phase response. (b) Differential phase simulations over total hemoglobin concentration; and (c) differential phase simulations over oxy-hemoglobin percentage. For (b) and (c), the steep slope region is tuned to the colon cancer of pre-malignant state ($C_{\mathrm{Hb}}(M) = 1.37 \times 10^{-4}$ and C_{ox} (%) = 51.3) [3] by adjusting amplitude ratio R and phase difference dP of two lasers to 1.47° and 181°, respectively. In the simulation, 680 nm and 780 nm lasers were square-wave modulated at 3.5 MHz

tumor state analysis since the amplitude does not exhibit the steep slope trend which is required for maximizing system sensitivity to pre-malignant tumors.

4 Experimental Apparatus and Procedures: Single-Laser Preliminary Measurement Toward WM-DPAS Detection

As a preliminary measurement for the WM-DPAS detection, PA studies using a single-laser source and a black-anodized metal have been performed. Obtaining the correct waveform is important in terms of validating the developed WM-DPAS theory since the differential phase value would rise from these waveforms. As depicted in Fig. 2a, a 800 nm CW laser (Laser Light Solutions 8800) was square-wave modulated at 0.3 MHz and was directed to the black-anodized metal immersed in water. The generated PA signal was measured with a 1 MHz focused transducer (Panametrics V314) and was then processed with a Labview software lock-in amplifier and osilloscope. A semi-infinite black-anodized metal is an ideal sample for the preliminary measurement since it has a high absorption coefficient and undergoes no deformation during the measurement. An assembled experimental setup illustrating future WM-DPAS measurements is described in Fig. 2b. In this setup, two laser diodes ($\lambda_A = 680\,\text{nm}$ and $\lambda_B = 808\,\text{nm}$) are directed to the same point of circulating sheep blood of which the oxygenation level is varied using sodium dithonite [4].

5 Results and Discussion

In Fig. 2c, preliminary single-frequency experimental results were obtained and compared to a developed theoretical formalism, which describes the PA signal generated by a square optical pulse as follows:

$$P(t) = \frac{I_0 \beta c_\text{a} \text{e}^{-\mu_\text{eff} L_1}}{C_p} \left[H\left(\frac{\tau_0}{2} - t\right) - \text{e}^{-\mu_\text{a} c_\text{a} t} + \text{e}^{-\mu_\text{a} c_\text{a} \left(t - \frac{\tau_0}{2}\right)} H\left(t - \frac{\tau_0}{2}\right) \right];\ 0 \le t \le \tau_0, \tag{6}$$

where I_0 is the laser intensity, β is the isobaric volume thermal expansivity of the absorber, C_p is the specific heat capacity at constant pressure, c_a is the speed of sound in the absorbing medium, μ_eff is the effective attenuation coefficient of the scattering medium, μ_a is the absorption coefficient of the absorber, L_1 is the depth of the absorber below the surface of the scattering medium, τ_0 is the modulation period, and $H(y) = \begin{cases} 1;\ y > 0 \\ 0;\ y < 0 \end{cases}$ is the Heaviside step function. Figure 2c shows the good agreement between the theoretical waveform from Eq. 6 and the experimental response. Some parameters assumed for best-fitting the theoretical response waveform to the theory include $c_\text{a} = 1.57 \times 10^5\,\text{cm}\cdot\text{s}^{-1}$ and $\mu_\text{a} = 9\,\text{cm}^{-1}$.

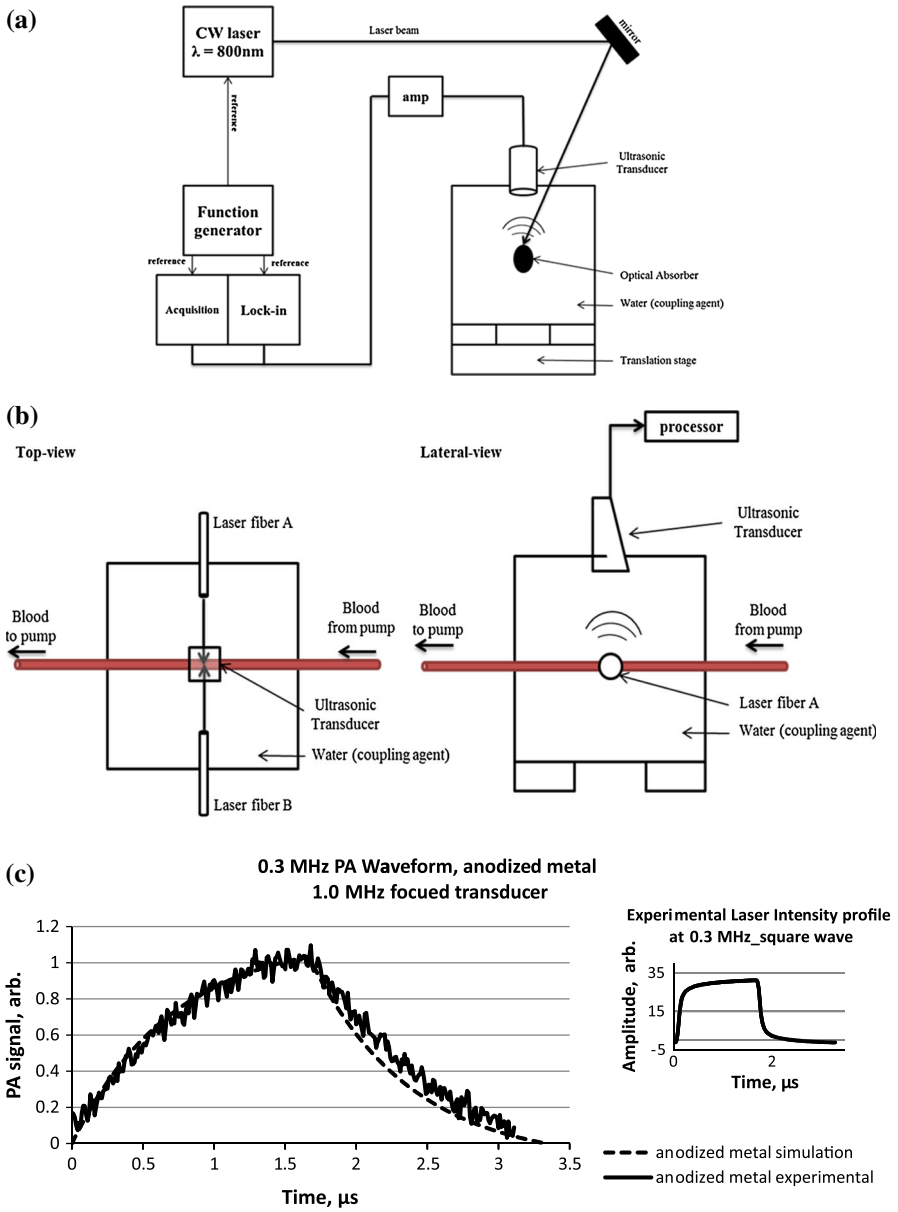

Fig. 2 Experimental setup: (a) single-ended PA setup schematic, (b) wavelength-modulated differential PA (WM-DPAS) setup schematic, and (c) single-laser PA study using 0.3 MHz square-wave input; theoretical PA waveform response of anodized metal (*dashed line*) and experimental PA waveform response of the anodized metal (*solid line*). Key parameters for the theoretical PA waveform include $c_a = 1.57 \times 10^5$ cm·s^{-1} and $\mu_a = 9\,\text{cm}^{-1}$. The experimental laser intensity profile is shown in the *inset*

6 Conclusions

WM-DPAS was theoretically developed and simulated, showing high sensitivity for early breast tumor detection as measured by its very high sensitivity to the degree of oxygenation of blood hemoglobin. A preliminary experimental PA response in the limit of single-laser square-wave excitation showed excellent agreement with the developed theoretical formalism in the same single-laser limit. WM-DPAS is expected to differentiate pre-malignant tumors from normal cells based on optical property differences before they can be measured by conventional single-laser PA detection.

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References

1. S. Telenkov, A. Mandelis, B. Lashkari, M. Forcht, J. Appl. Phys. **105**, 102029 (2009)
2. S. Telenkov, R. Alwi, A. Mandelis, A. Worthington, Opt. Lett. **36**, 4560 (2011)
3. H. Wang, J. Jiang, C. Lin, J. Lin, G. Huang, J. Yu, Opt. Express **17**, 4 (2009)
4. K. Briley-Sæbø, A. Bjørnerud, Proc. Int. Soc. Magn. Reson. Med. **8**, 2025 (2000)