Photoacoustics 2 (2014) 1–11

Contents lists available at ScienceDirect

Photoacoustics

journal homepage: www.elsevier.com/locate/pacs

Research Article

Nonlinear photoacoustic signal amplification from single targets in absorption background

Mustafa Sarimollaoglu *, Dmitry A. Nedosekin, Yulian A. Menyaev, Mazen A. Juratli, Vladimir P. Zharov

Phillips Classic Laser and Nanomedicine Laboratories, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AR 72205, USA

ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form 30 October 2013
Accepted 7 November 2013

Keywords:
Photoacoustic imaging
Flow cytometry
Nonlinear phenomena
Nanobubbles
Circulating tumor cells
Melanoma

ABSTRACT

Photoacoustic (PA) detection of single absorbing targets such as nanoparticles or cells can be limited by absorption background. We show here that this problem can be overcome by using the nonlinear photoacoustics based on the differences in PA signal dependences on the laser energy from targets and background. Among different nonlinear phenomena, we focused on laser generation of nanobubbles as more efficient PA signal amplifiers from strongly absorbing, highly localized targets in the presence of spatially homogenous absorption background generating linear signals only. This approach was demonstrated by using nonlinear PA flow cytometry platform for label-free detection of circulating melanoma cells in blood background *in vitro* and *in vivo*. Nonlinearly amplified PA signals from overheated melanin nanoclusters in melanoma cells became detectable above still linear blood background. Nonlinear nanobubble-based photoacoustics provide new opportunities to significantly (5–20-fold) increase PA contrast of single nanoparticles, cells, viruses and bacteria in complex biological environments.

1. Introduction

Photoacoustic (PA) spectroscopy, cytometry, and imaging demonstrated tremendous potential for high sensitivity detection, visualization, and identification of nanoparticles (NPs), cells, vessels and other absorbing targets *in vitro* and *in vivo* [1–9]. In particular, PA flow cytometry (PAFC) demonstrated detection of single circulating tumor cells (CTCs), cancer stem cells, pathogens, clots, normal and abnormal (*e.g.*, sickle) blood cells as well as pharmacokinetics of NPs, capsulated dyes, and drug carriers in blood, lymph, bone, cerebral, and plant vasculatures [9]. In many applications, especially in biomedicine, PA detection limit can be restricted by background from absorption medium such as cellular cytoplasm, biotissue or blood. Many approaches were proposed to reduce the influence of absorption background due to the presence of multiple absorbing components in medium, including: (1) generation of second harmonic of PA signals from absorbing molecules with saturated absorption in the presence of linear background [2,10]; (2) increased PA contrast from targets with multiphoton absorption compared to background with linear absorption [11]; (3) two beam excitation with different wavelength and modulation frequency and detecting PA signals at frequency difference [2,12]; (4) discrimination of targets with different temperature-dependent absorption by sample heating or cooling down to liquid nitrogen temperature [2,12]; (5) discrimination of targets with different relaxation time [13]; (6) blood oxygenation and deoxygenation [14]; (7) magnet-induced PA signals preferentially from magnetic NPs only [15]; (8) laser-induced generation of nanobubbles as significant PA signal amplifier around strongly absorbing, spatially localized targets in relatively homogenous absorption background with no nanobubble formation [8]. The last approach was demonstrated to improve detection limit or enhance PA imaging contrast for quantum dots [16], melanoma cells in blood [2,14], golden carbon nanotubes in lymph [17,18], bacteria with intrinsic pigment (carotenoids) and silica-magnetic NPs in blood [19], and carbon nanotubes in plants [20]. Simultaneously with PA contrast enhancement, laser-induced nanobubbles led to spectral and spatial sharpening of PA and photothermal (PT) phenomena that allowed to break spectral (up to 1 nm) and diffraction (up to 50 nm) limits [8,21]. Nanobubble generation thresholds demonstrated high sensitivity to NP clustering (*e.g.*, larger nanocluster size – lower threshold level) that was used in PT-based

* Corresponding author. Tel.: +1 501 526 7620.
E-mail address: MSarimollaoglu@uams.edu (M. Sarimollaoglu).

2213-5979
http://dx.doi.org/10.1016/j.pacs.2013.11.002

nanodiagnosis and nanotherapy [22]. PA spectroscopy and imaging was also explored using other nonlinear phenomena such as the dependence of PA signal amplitude on temperature-dependent target or medium parameters [23–26], photochemical reaction [27], multiphoton absorption [28], and absorption saturation [29]. Nevertheless, the detailed analysis of nonlinear detection of single NPs and cells in absorption background is lacking.

Here we fill this gap by exploring potential of nonlinear PAFC for detecting single CTCs in blood. Clinical significance of CTCs is associated with metastasis as leading cause of cancer deaths (50 reviews were published in 2012 alone and 300 clinical trials are in progress) [30–35]. Metastasis is the results of CTCs spreading from primary tumor to distant organs through blood network. Clinical studies have demonstrated the tremendous potential for using CTC count as a marker of metastatic development, cancer recurrence, and therapeutic efficacy [31]. Despite significant progress in development of CTC assays, the existing assays *ex vivo* still have many limitations including time-consuming blood processing and low sensitivity due to small blood samples [30–35]. As a result, at the time of initial detection, incurable metastasis might already have developed. To overcome these problems, *in vivo* PAFC was developed with the capability for detecting single CTCs in whole blood volume circulating in the peripheral blood vessels [9,14,17]. In particular, label-free detection of melanoma CTCs was demonstrated using melanin NPs as intrinsic melanoma marker [14]. Although cutaneous melanoma is the third most common type of skin cancer and accounts for only 3% of all cases, it accounts for 65% of all deaths from skin cancer [33]. The most alarming aspect of melanoma is its potential to metastasize at a very early stage of the disease. A pre-clinical study with PAFC revealed the feasibility of label-free melanoma CTC detection *in vivo* at extremely low concentration of 1 CTC/mL [9,14]. Most melanoma cells contain a large amount of melanin, a natural pigment with strong optical absorption in a wide spectrum in visible and near-infrared (NIR) range [14]. This makes PA techniques, an almost ideal method for label-free detection of strongly pigmented melanoma CTCs [14,32], especially *in vivo*. However, conventional linear mode PAFC can miss low pigmented CTCs which produce PA signals below blood background [36]. We demonstrate here that next generation of PAFC can solve this problem by nonlinear amplification of PA signals from laser-induced nanobubbles even in rare melanin NP clusters in low pigmented cells.

2. Materials and methods

2.1. Principle of nonlinear PAFC

When a CTC with intrinsic absorbing markers such as melanin or exogenous labels is illuminated by laser pulses, the absorbed energy is converted to heat which in turn induces thermal expansion of the heated zones leading to generation of PA waves referred as PA signals (Fig. 1a). Amplitude of a PA signal in linear mode is proportional to laser energy and absorption coefficient of the target. In positive-contrast PAFC, signal-to-background ratio (SBR) is determined by the ratio of PA signal amplitude from single CTC to background signal amplitude which is superposition of signals from individual red blood cells (RBCs) in the detected volume and noise of different origins (*e.g.*, electronic, acoustic, fluctuating RBC number, or instability of laser energy [typically 3–5%]). According to multiple verifications using *in vivo* mouse model and *ex vivo* human blood spiked with melanoma cells, PAFC in near-infrared (NIR) spectral range (*e.g.*, at 820 nm or 1064 nm) can detect single pigmented melanoma CTC in the presence of ~100–300 RBCs that is also in line with coefficients of absorption for blood and melanin (see details in Ref. [14]). However, linear PAFC can miss low pigmented cells in blood background [36]. This

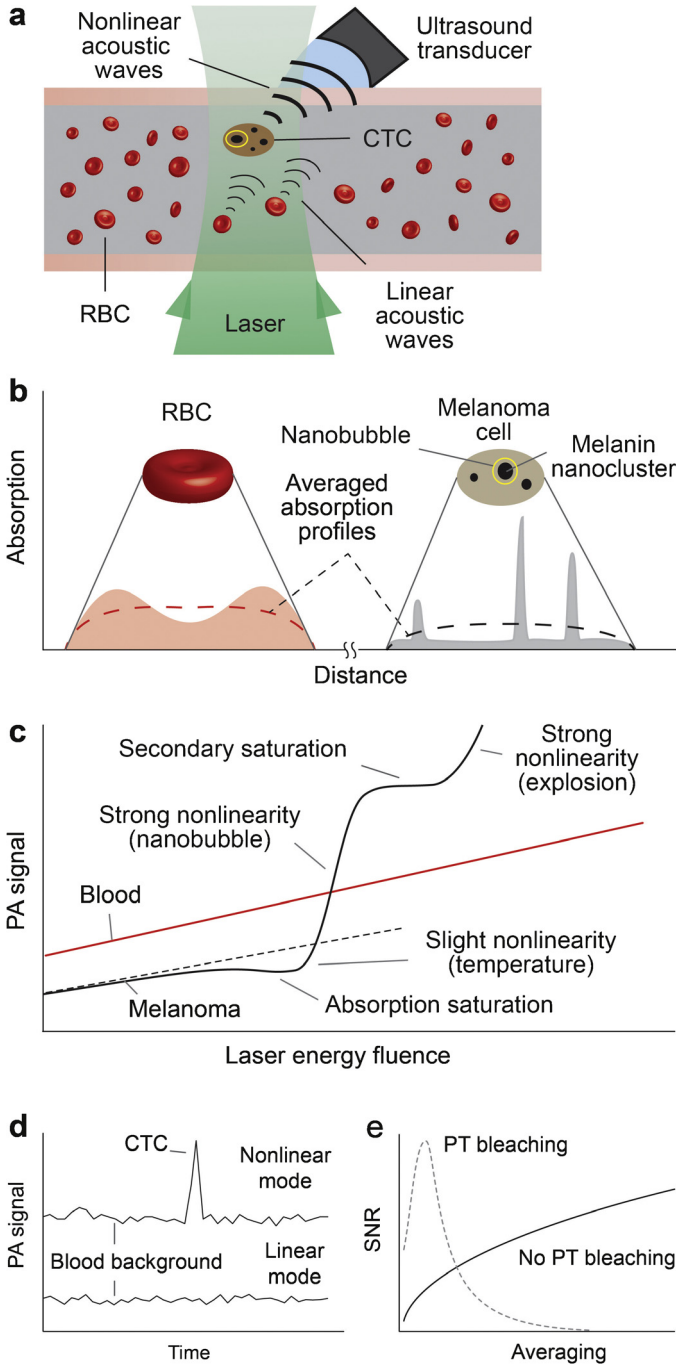

Fig. 1. Principle of nonlinear PAFC. (a) Schematics. (b) Absorption profiles of an RBC and a melanoma cell. (c) Linear and nonlinear PA signal dependence on energy fluence from RBCs and melanoma cells. (d) PA signal traces in linear and nonlinear modes. (e) Dependence of signal-to-noise ratio (SNR) on averaging in the presence of photothermal bleaching.

problem can be partly overcome in nonlinear PAFC based on the differences in PA signal dependences on laser energy from targets and background. According to previous findings [8,9,16–21,26,29,37] and phenomenological model [8], absorbing targets (*e.g.*, NPs, dyes, and chromophores) exhibit multistage behaviors when the energy fluence (E) is increased (Fig. 1c, black curve): (1) A gradual linear increase at a low fluence, E^n ($n = 1$); (2) absorption saturation ($n \sim 0.5$–0.9); (3) slight nonlinear signal increase ($n \sim 1.2$–1.6) related with temperature-dependent thermophysical parameters; (4) strong nonlinear signal amplification ($n \sim 2$–5) related with nanobubble formation and its spatial and temporal