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Noninvasive Determination of Melanoma Depth using a Handheld Photoacoustic Probe

TO THE EDITOR

When a clinically suspicious pigmented lesion is biopsied and histologic examination shows melanoma, tumor depth, or Breslow's depth (BD), is the key parameter that both determines surgical margins for definitive excision and serves as an indication to perform sentinel lymph node biopsy (Smith and MacNeil, 2011). Optimally, BD is measured during an initial excisional biopsy that includes the entire lesion. However, in many cases an incisional biopsy is performed that takes a sample of only a portion of the tumor, possibly resulting in an inaccurate measurement (Guitera and Menzies, 2011; Sellheyer et al., 2010). Wide local excision based on a potentially inaccurate BD may be insufficient to adequately treat the tumor, necessitating a second definitive surgery. Thus, a precise in vivo measurement of tumor depth would facilitate appropriate surgical treatment at the time of wide local excision when only a partial biopsy sample has been obtained.

In this work, we propose applying photoacoustic tomography (PAT) to address this problem. Compared with traditional optical imaging methods, PAT uses ultrasonic waves, which give approximately 1,000 times less scattering than optical waves, breaking through the optical diffusion limit ($\sim$1 mm in the skin) for penetration (Wang and Hu, 2012; Wang and Gao, 2014). In addition, by using optical excitation of acoustic waves due to light absorption, PAT provides improved contrast of melanin, which is deficient in ultrasonographic imaging. In previous mouse models, we measured a melanoma greater than 7 mm in depth (Zhou et al., 2015). Here we extend our work to patients with melanoma, with the aim of determining the accuracy of PAT-measured melanoma depth compared with the actual BD based on excisional biopsy results. In addition, we also compare our PAT measurement with the histologic measurement obtained from partial incisional biopsy to ultimately determine the predictability and application of PAT measurements in a clinical setting.

We applied a linear array-based handheld photoacoustic probe (Figure 1) (LZ250, Visualsonics Inc., Toronto, Ontario, Canada) for melanoma imaging in patients (Hai et al., 2015; Needles et al., 2013). The transducer array has 256 elements with a size of 23 mm × 3 mm. Each element in the array is cylindrically focused at 15 mm distance. With a central frequency of 21 MHz and a 70% one-way bandwidth, the probe provides spatial resolutions of 1,237 μm, 119 μm, and 86 μm in the elevational, lateral, and axial directions, respectively (Zhou et al., 2015). For the highest melanoma detection sensitivity, pulse-light at 680 nm illuminates the target, where blood has low absorption (Yao and Wang, 2014). The optical fluence on the skin surface is approximately 10 mJ/cm^2, which is less than the safety limit set by the American National Standards Institute (20 mJ/cm^2) at this wavelength.

Our study was approved by the Institutional Review Board of Washington University in St. Louis (IRB ID#: 201410125). Ten patients were recruited, and 13 lesions were imaged. Benefits and risks of the study were explained to the patients in detail, and written consent was obtained. We initially performed PAT imaging of the melanomas using our handheld probe followed by an excisional biopsy to determine the BD of each melanoma. Of the 13 lesions that were imaged, six presented after an initial incisional biopsy, providing a provisional BD (pBD) before PAT imaging (See Supplementary Table S1 and S2 online). After imaging and histologic examination, we determined that one lesion was composed entirely of melanophages with no tumor cells, and another amelanotic tumor extended to a depth (>10 mm) that was beyond the maximum imaging capacity of the PAT probe, although the most superficial portion of the tumor could be detected. A third patient who presented with widely metastatic disease received systemic therapy at an outside institution before wide local excision of his primary lesion. Thus, we excluded the lesions from these three patients from our final data analysis.
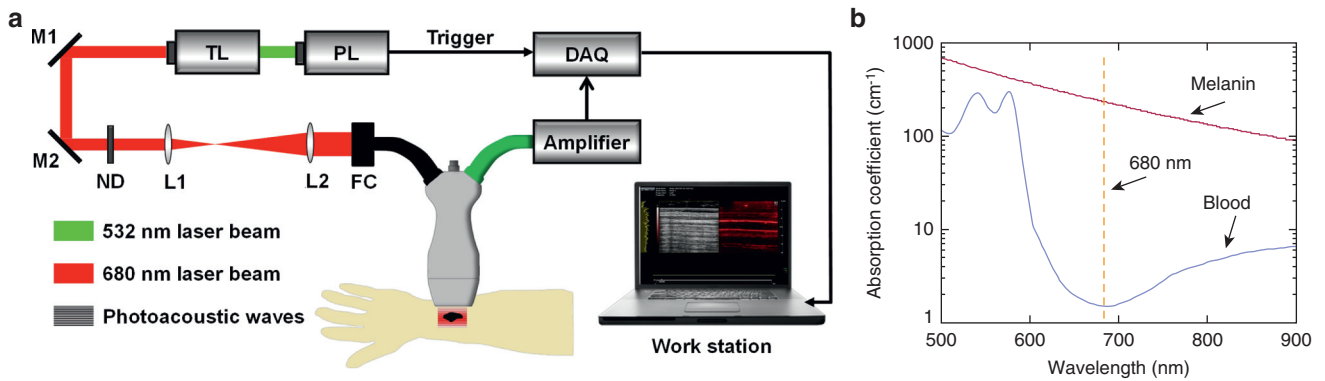

Figure 1. Photoacoustic system for imaging in melanoma patients. (**a**) Schematic of the photoacoustic system. DAQ, data acquisition system; FC, fiber coupler; L1 and L2, lenses; M1 and M2, mirrors; ND, neutral density filter; PL, pump laser; TL, tunable laser. (**b**) Absorption spectra of melanin and blood at typical concentrations in tissue. A wavelength of 680 nm was chosen for melanoma imaging, at which the absorption ratio between melanin and blood is relatively high.
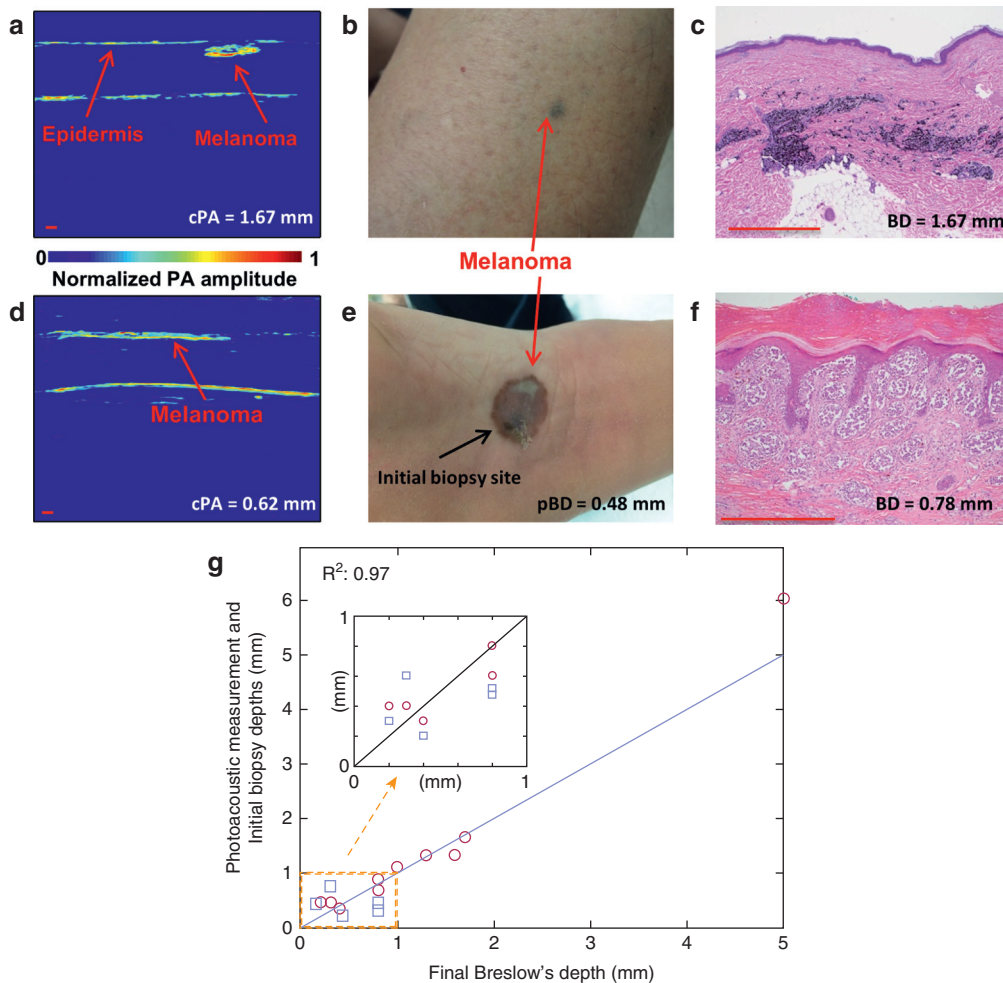

Figure 2. PAT-based depth is more accurate than the provisional incisional biopsy depth (pBD). PAT of melanoma two patients: (**a–c**) a patient with cutaneous melanoma metastases on the left lower extremity and (**d–f**) a patient with primary acral lentiginous melanoma on the right foot. (**a**) Melanoma image acquired with PAT, clearly showing the melanoma and skin surface with a PA depth of 1.9 mm (cPA depth = 1.67 mm). Scale bar = 1 mm. (**b**) Cutaneous melanoma metastasis in a patient's lower leg. (**c**) Histology of the excised melanoma, showing actual BD of 1.67 mm. Original magnification ×40. Scale bar = 1 mm. (**d**) PAT melanoma image of the acral lentiginous melanoma with PA depth of 0.70 mm (cPA depth = 0.62 mm). Scale bar = 1 mm. (**e**) Acral lentiginous melanoma (data not shown: pBD = 0.48 mm). (**f**) Histology after complete excision, actual BD of 0.78 mm. Original magnification ×100. Scale bar = 0.5 mm. (**g**) cPA depth (red circles) and pBD (blue squares) plotted against actual BD. Blue line shows 1:1 concordance of PAT measurements (cPA) if identical to the actual BD after excision. Inset graph shows cPA (red circles) and pBD (blue squares) plotted against a 1:1 concordance of pBD to actual BD (black line). BD, Breslow's depth; cPA, corrected photoacoustic tomography; PAT, photoacoustic tomography; pBD, provisional Breslow's depth.

PAT images from a cutaneous melanoma metastasis on the left lower extremity (Figure 2a) and a primary cutaneous acral lentiginous melanoma (Figure 2d) clearly show the ability of PAT to detect dermal and epidermal melanin-containing components of the tumors, allowing for direct in vivo measurement of melanoma depth. (PAT measurement was adjusted by a factor of 0.88 to account for ex vivo tissue fixation and processing [Winsor, 1994], and this is indicated as corrected [cPA] depth.) Immediately after imaging, an excisional biopsy was performed and actual BD was determined (Figure 2c and f). As shown in Figure 2a−c, the cutaneous melanoma metastasis PAT depth in vivo (cPA) is concordant with the actual BD. Similarly, the primary tumor cPA measurment more closely represented the acutal BD than the pBD obtained from a partial biopsy (Figure 2d−f).

The depth of all of the tumors that were successfully imaged ranged between 0.2 mm and 6.0 mm (Figure 2g). cPA depths are concordant with actual BD obtained after excisional biopsy (mean absolute error (MAE) = 0.18 mm; R^2 = 0.97). When comparing cPA obtained by PAT with the actual BD in the five patients with primary melanomas who had incisional biopsy before PAT, PAT achieved accurate measurements (MAE = 0.14 mm). Comparing cPA measurement with the incisional biopsy pBD is less concordant, as might be expected (MAE = 0.21 mm). Thus, in our pilot study, PAT was accurate in determining BD in melanoma and was an effective imaging tool that accurately determined melanoma depth to potentially guide definitive surgical intervention.

In this pilot study with a limited number of patients, PAT did not alter but rather confirmed current surgical treatment based on pBD, particularly for standard pigmented melanomas compared with amelanotic melanomas. PAT provided an accurate measurement of melanoma depth as determined by BD measurement of the residual tumor on excisional biopsy. We acknowledge that melanomas with a depth of 10 mm or greater were beyond the detection limit of our current system because of strong light attenuation resulting in weak signal. Although we have addressed this technical problem in previous systems (Zhou et al., 2014), this technology was not incorporated into our non-handheld prototype because of the technical design of the probe. In this current handheld system, we conclude that PAT technology is noninvasive, provides high contrast for melanoma imaging with detection capabilities, and adapts easily to bedside monitoring, providing information that may potentially facilitate early and effective surgical intervention.

CONFLICT OF INTEREST
The authors state no conflict of interest. LVW has a financial interest in Microphotoacoustics, Inc., which, however, did not support this work.

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