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# Photoacoustic imaging of single circulating melanoma cells *in vivo*

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## ABSTRACT

Melanoma, one of the most common types of skin cancer, has a high mortality rate, mainly due to a high propensity for tumor metastasis. The presence of circulating tumor cells (CTCs) is a potential predictor for metastasis. Label-free imaging of single circulating melanoma cells *in vivo* provides rich information on tumor progress. Here we present photoacoustic microscopy of single melanoma cells in living animals. We used a fast-scanning optical-resolution photoacoustic microscope to image the microvasculature in mouse ears. The imaging system has sub-cellular spatial resolution and works in reflection mode. A fast-scanning mirror allows the system to acquire fast volumetric images over a large field of view. A 500-kHz pulsed laser was used to image blood and CTCs. Single circulating melanoma cells were imaged in both capillaries and trunk vessels in living animals. These high-resolution images may be used in early detection of CTCs with potentially high sensitivity. In addition, this technique enables *in vivo* study of tumor cell extravasation from a primary tumor, which addresses an urgent pre-clinical need.

**Keywords:** Photoacoustic imaging, circulating tumor cell, melanoma

## 1. INTRODUCTION

Melanoma is one of the most common types of skin cancer. As in many other types of cancer, the major challenge in treating melanoma is tumor metastasis, which is also one of the key reasons that this type of cancer has a high mortality rate. Circulating tumor cells (CTC) is one of the key predictors for tumor metastasis(1). *In vivo* imaging of rare CTCs may help early diagnosis of tumor metastasis and provides a powerful tool to study the fundamental mechanism of tumor cell extravasation from the primary tumor.

Optical-resolution photoacoustic microscopy (OR-PAM) offers high spatial resolution, optical absorption contrasts, and functional imaging capabilities (2-5). In addition, for melanoma cells, photoacoustic (PA) techniques can provide label-free imaging with high sensitivity. All of these advantages make OR-PAM an ideal candidate to image circulating melanoma cells.

Here, we developed video-rate OR-PAM (6) for imaging of rare circulating melanoma cells *in vivo*. The imaging system has sub-cellular spatial resolution and works in reflection mode. A fast-scanning mirror allows the system to acquire fast volumetric images over a large field of view. A 500-kHz pulsed laser was used to image blood and CTCs. Single circulating melanoma cells were imaged in both capillaries and trunk vessels in living animals. These high-resolution images may be used in early detection of CTCs with potentially high sensitivity. In addition, this technique enables an *in vivo* study of tumor cell extravasation from a primary tumor, which addresses an urgent pre-clinical need.

## 2. IMAGING SYSTEM DESIGN

A fast pulsed laser (wavelength: 532 nm and 1064 nm, pulse width: 3 ps, 500 kHz) delivers light to the OR-PAM system(6). As shown in Figure 1, the laser beam is first focused by an optical objective, and then passes through an aberration-correction lens, and is reflected by a prism mirror. The reflected optical beam is further redirected onto the sample via a scanning mirror. The emitted spherical ultrasonic wave is firstly reflected by the same scanning mirror, and then collected by a concave acoustic lens. The acoustic lens converts the spherical ultrasonic wave into a planar wave, which passes through an optical/acoustic beam combiner, and is detected by a broadband ultrasonic transducer. The optical/acoustic beam combiner is made of two right-angle glass prisms with a thin layer of aluminum coating in the middle. Because aluminum coating has an acoustic impedance similar to that of glass, the beam combiner effectively transmits the ultrasonic wave and reflects the optical beam. The optical beam is precisely aligned with the ultrasonic beam, so that the imaging system can achieve high sensitivity. The scanning mirror can provide a one-dimensional (along the *x*-axis) fast mechanical scan in water, which maintains the alignment between the optical and acoustic beams. Another linear translational stage scans the animal in the elevational direction (*y*-axis). The imaging system provides fast imaging ability and high spatial resolution, which allows the detection of melanoma cells *in vivo*.

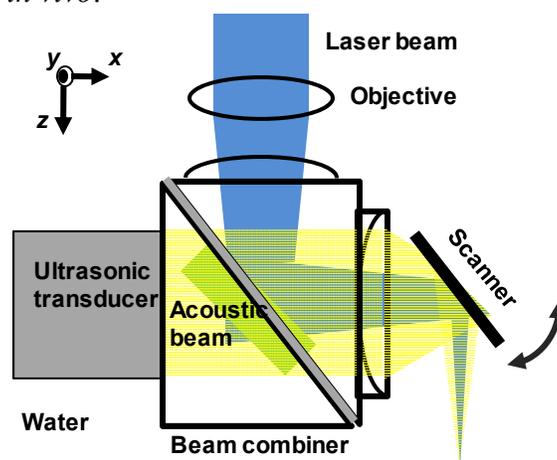


Figure 1. schematics of the fast-scanning OR-PAM system.

### 3. EXPERIMENTAL RESULTS

In order to validate the system performance, we first imaged a blood phantom mixed with melanoma cells in a rubber tube. Using 532nm wavelength, melanoma cells generated 200%~300% stronger photoacoustic signal than blood. With fast imaging ability and optically-defined lateral resolution, we can image single melanoma cells flowing with the blood.

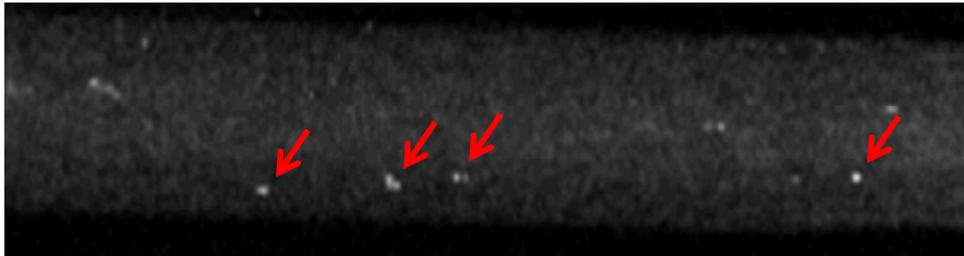


Figure 2. Imaging of melanoma cells in whole blood. Arrows point to melanoma cells

By injecting melanoma cells into the jugular vein of the mouse, we imaged circulating tumor cells *in vivo*. Figure 3 shows a melanoma cell passing a capillary vessel. The signal from the single melanoma cell is several times stronger than that from single red blood cells in the capillary. With fast imaging speed and high spatial resolution, we can also image single melanoma cells in bigger vessels. Figure 4 shows imaging of a circulating melanoma cell when it flows in a vein vessel. Control experiments had been carried out to validate that the strong signals were from melanoma cells.

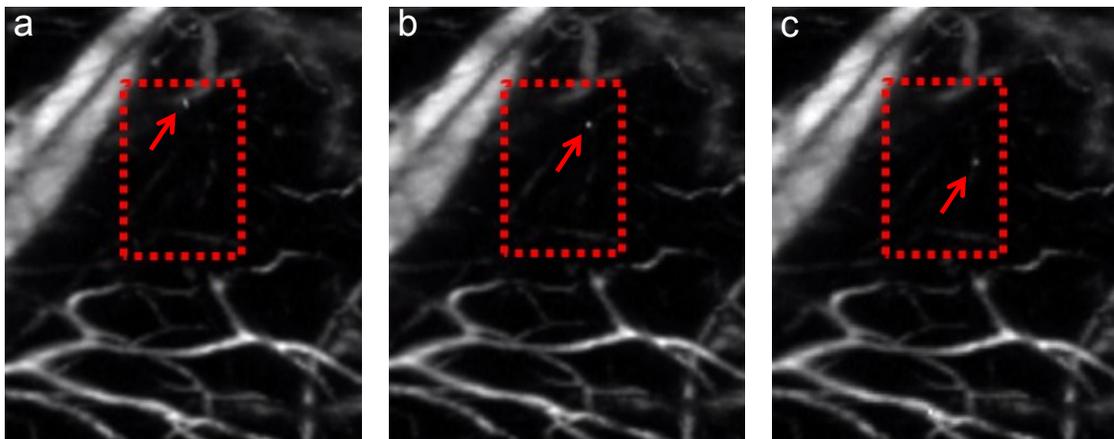


Figure 3. *In vivo* imaging of melanoma cell in capillary. Time interval between consecutive images is 1 seconds. Arrow pointing a melanoma cell passing through a capillary.

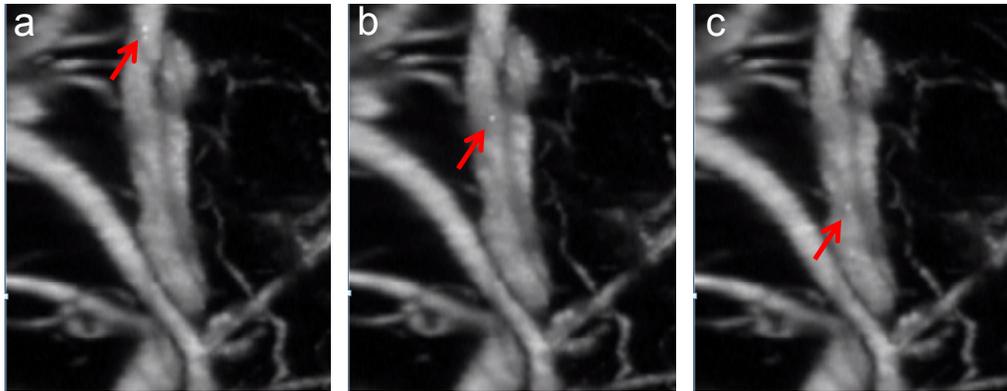


Figure 4. *In vivo* imaging of melanoma cell in vein. Time interval between consecutive images is 0.5 seconds. Arrow pointing a melanoma cell flow in a vein vessel.

#### 4. CONCLUSION

In summary, we have successfully developed video-rate OR-PAM for imaging of circulating melanoma cells *in vivo*. CTCs in both capillary and trunk vessels have been imaged in real time. Our system even allows us to image CTCs with near-infrared wavelength in the future, which should provide higher imaging contrast. This method may be used in early detection of CTCs with potentially high sensitivity and *in vivo* study of tumor metastasis mechanism.

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