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Abstract. Melanoma is the deadliest form of skin cancer, yet current diagnostic methods are unable to detect early onset of metastatic disease. Patients must wait until macroscopic secondary tumors form before malignancy can be diagnosed and treatment prescribed. Detection of cells that have broken off the original tumor and travel through the blood or lymph system can provide data for diagnosing and monitoring metastatic disease. By irradiating enriched blood samples spiked with cultured melanoma cells with nanosecond duration laser light, we induced photoacoustic responses in the pigmented cells. Thus, we can detect and enumerate melanoma cells in blood samples to demonstrate a paradigm for a photoacoustic flow cytometer. Furthermore, we capture the melanoma cells using microfluidic two phase flow, a technique that separates a continuous flow into alternating microslugs of air and blood cell suspension. Each slug of blood cells is tested for the presence of melanoma. Slugs that are positive for melanoma, indicated by photoacoustic waves, are separated from the cytometer for further purification and isolation of the melanoma cell. In this paper, we evaluate the two phase photoacoustic flow cytometer for its ability to detect and capture metastatic melanoma cells in blood. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.6.061221]

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1 Introduction

It is estimated that over 70,000 Americans will be diagnosed with melanoma in 2011 and nearly 9000 melanoma patients will die.1 While melanoma can be clinically managed if detected early and removed, if it metastasizes, the cancer can become lethal.2,3

In metastatic disease, circulating tumor cells (CTCs) separate from primary or secondary tumors and move through the blood or lymphatic system, planting themselves elsewhere and creating secondary tumors that are subsequently the cause for terminal cancer.2,4 Being able to detect the CTCs in blood will allow clinical interventions to have a greater chance of success. There is some disparity in the literature as to the levels of CTCs in patients with metastatic cancer, ranging from 2 CTCs per 7.5 mL of blood1 to hundreds of CTCs per 1 mL of blood.5 Regardless, these numbers are orders of magnitude less than other components of whole blood which measure 10^6 cells/mL, requiring very sensitive detection methods to find these rare cells. In addition, researchers have discovered a positive correlation between the number of CTCs present in blood and the prognosis of the patient, rendering information regarding the number of CTCs useful in monitoring therapy. Hypothetically, low CTC numbers indicate that treatment is working well, while high numbers indicate otherwise. Obtaining pure isolates of CTCs not only precludes false positives, but also provides cancer biologists with early stage CTCs to study.

The detection, isolation, and characterization of CTCs can provide insight into disease status and can be used to monitor the response to therapy. Furthermore, molecular characterization of CTCs can provide fundamental insight into how cells leave a primary tumor and establish distant metastases. A metastatic cell must leave the primary tumor and enter either the lymphatic system or the vasculature. Subsequently, the metastatic cell must invade a distant tissue and proliferate to form a metastatic tumor. Thus, the cells in a metastasis are derived from cells that were, at one time, CTCs. However, the molecular characteristics of a CTC that enable it to seed sites of metastatic tumor growth are largely unknown.

Therefore, there exists a clinical need to detect the presence of circulating melanoma cells in blood, to count their number, and to obtain isolates of these cells that are free from other cells.3 It is also desirable that these objectives be achieved in as short a time as possible without subjecting the target cells to physical or chemical processes that may affect their molecular biology.

Current methods for diagnosing metastatic cancer include lymph node biopsies and imaging techniques.8 Lymph node biopsies can produce false negatives if the cancer did not interact with the lymphatic system and cannot be performed numerous times for disease monitoring.8 Imaging techniques require the presence of tumors large enough to appear on scans before cancer can be diagnosed. Considering that a 1 mm diameter tumor typically consists of millions of cells, larger more visible tumors can generally consist of billions of cells.10 Therefore, a patient who is diagnosed with a large tumor will have reduced options and will require more physically demanding treatment than a...
patient who is diagnosed before macroscopic secondary tumors are detected.

CTCs can be an excellent source of information for diagnosis, and may enable clinicians to monitor metastatic disease. Many techniques are being investigated to detect and isolate these cells for diagnostic, monitoring, and exploratory purposes. Some research has already been conducted to detect CTCs, including RT-PCR, immunohistochemistry, magnetic cell sorting, optical scanning systems, lab on a chip devices, and microfilters, yet high false negative rates, labeling, long procedures, and highly complex devices limit chances of clinical implementation.6,11–17

Other methods involve the use of flow cytometry, which has been utilized by numerous groups to detect CTCs.18–23 Traditional flow cytometry measures size, granularity, and relative fluorescence intensity. Furthermore, in traditional flow cytometry only one cell can flow past the laser beam at a time. Flow cytometry uses fluorescent signals from labeled cells to classify pathological and normal cells. These signals are weak and flow cytometry is not traditionally used for detecting rare events, such as CTCs from solid tumors. In the case of so called liquid tumors such as leukemia, cancer cells may constitute as much as 5 to 10% of all leukocytes. Thus, good statistical information is obtained with insensitive sampling. In order to detect rare CTCs, however, a more robust optical signal must be used in flow cytometry. A purely optical set-up that measures purely absorption would require that only one cell be evaluated at a time because scattering and absorption effects are not distinguishable in transmission measurements, and cell clumping would generate ambiguous results.

We have developed a photoacoustic flow cytometer that induces high frequency ultrasonic waves in pigmented cells as a means of detecting circulating melanoma cells in blood samples. Our system detects pigmentation due to thermoelastic expansion when an absorber is irradiated with a rapid pulse of light. Colorless particles, such as white blood cells, remain acoustically transparent allowing numerous cells to cross the detection beam at a given time.21–23 Zhavor et al. have also performed flow cytometry with photoacoustic detection in vivo on a nude mouse ear and have enriched the sample in vivo using magnetic nanoparticles.19,20,24 While these techniques overcome great technical challenges, the in vivo nature of the method severely complicates the process of capturing the detected cells. In order to capture the detected cells from the blood stream, the vein or capillary where detection occurred would have to be shunted, an invasive procedure that would limit clinical monitoring of a cancer patient. In addition, due to laminar flows velocity profile, it would be impossible to predict where the cell would be after detection. Any attempt at capturing in vivo would likely have to employ accumulation of cells with the aid of magnetic attraction and magnetic nanoparticle labeling which negates the entire attractiveness of the label free detection offered by the photoacoustic method.

We have incorporated two phase flow into our in vitro flowmetry technique in order to increase the certainty of where a cell is located within the system.11–14 It is well known that two immiscible fluids, such as air and water, do not mix and it has been observed that isolated slugs of each fluid phase can be created under microfluidic conditions when confluent at a T-junction with conditions of low Reynolds and Capillary (Ca) numbers.25–28 For a given set of flow rates, the sizes of the slugs remain constant and the total flow rate of the mixture is the sum of the flow rates. As each slug flows past the photoacoustic detector, it is tested for optical absorbers, and since the flow rate of the fluid mixture is known, the positive droplets can be tracked and separated from the rest of the sample. After initial capture of positive slugs, the isolates are diluted using PBS and rerun through the photoacoustic flow system. The volume is again compartmentalized and positive slugs are once more identified and captured. This process continues until only melanoma cells exist in suspension with PBS.

It is believed that under microfluidic conditions, surface tension becomes the dominating force on fluid flow. The alternating droplets are generated due to a more thermodynamically stable system than the alternative, which consists of two fluids flowing side-by-side. Prior studies have indicated that for the alternating flow to be generated simultaneously, the Ca of the system must be less than 0.01.28 The Ca for a gas-liquid system is given by the relation:

\[ Ca = \frac{\mu \nu}{y}, \]

where \( \mu \) is the viscosity of the liquid, \( \sigma \) is a characteristic velocity, and \( y \) is the surface tension between the liquid and the gas. The Ca is a ratio of the relative strengths of the viscous forces to the surface tension forces. A value significantly lower than one implies that surface-tension forces are stronger, and therefore govern the behavior of the system.

The main advantage over single phase flow systems is that confining small amounts of a particular fluid between regions of another fluid with which it is immiscible enables tracking of the material contained within a single slug. In single phase flow systems, Taylor dispersion, a phenomenon that dictates the velocity profile of fluid through a conduit, must be taken into account. Since the velocity is not uniform, it is impossible to predict the exit time of a cell traveling in solution. By confining small amounts of a particular fluid between regions of an immiscible fluid, two phase flow allows tracking of the material contained within a single droplet, eliminating complexities brought about by Taylor dispersion.

Some groups have already shown that encapsulation of cells is possible;29,30 however, this report shows the first use of two phase flow to isolate pigmented cells as a test for CTCs. The generation of slugs allows for the compartmentalization of detected melanoma cells and extraction in small volumes from the flow system.

We report sensitivity and selectivity experiments for our photoacoustic flow cytometer, as well as demonstrate the microfluidic cell capture technique.

2 Materials and Methods

We designed and tested a photoacoustic flow cytometer in order to evaluate its suitability for use in sensing CTCs. The clinical implementation would entail obtaining approximately 10 mL of whole blood from a patient at risk for or having metastatic melanoma. The white blood cells are separated from the sample using standard centrifugation. Any circulating melanoma cells (CMCs) present would separate in the white blood cell layer due to similar density. The white blood cells are resuspended in 10 mL of phosphate buffered saline (PBS) and injected into the photoacoustic flow cytometer.

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2.1 Flow Chamber Design

A single cell is considered an optical point source and will therefore emit photoacoustic waves radially. The flow chamber, shown in Fig. 1, was held together with an acrylic ring that had three holes drilled at 90 deg from each other. Masterflex tubing was fed through the holes and a wire with the same outer diameter as the tubing’s inner diameter was suspended through the two opposing holes while a second wire was fed through the third hole to prevent acrylamide from entering the tubing hole.

Once the acrylic ring was prepared, Parafilm was stretched across the bottom of the ring and clear acrylamide was poured into the ring, gelling around the tubing and wire. The acrylamide was made from 10 mL of acrylamide solution [20 g acrylamide (Sigma Aldrich), 0.7 g bisacrylamide (Sigma-Aldrich, St. Louis, Missouri), 100 mL of distilled water], 0.04 g of ammonium persulfate (Sigma-Aldrich, St. Louis, Missouri) and 20 μL of Tetramethyl ethylenediamine (TEMED) (Fisher Scientific, Pittsburgh, Pennsylvania). After adding the TEMED, the mixture was poured immediately to avoid premature gelling.

After gelling, the wires were removed and the third tube was used to hold the optical fiber in place, aiming at the flow path. The flow chamber was then ready to be used in the system.

2.2 Sample Preparation

An HS 936 melanoma cell line was cultured for use in photoacoustic experiments and cells were counted using a hemocytometer. Approximately 15 min before experiments, the cells were diluted with PBS and 2% Tween 20 to the desired concentration of melanoma cells per microliter.

2.3 Photoacoustic Flow System

Photoacoustic flowmetry is similar to classic flow cytometry; a sample of interest is directed past a detector to identify if a certain analyte is present. Unlike flow cytometry, our apparatus allows millions of cells to flow past the detector simultaneously due to the photoacoustic transparency of white blood cells, allowing large volumes to be scanned very quickly for CMCs.

![Fig. 1 The flow chamber for the photoacoustic flowmeter consists of a flow path in an acrylamide mold that allows cell suspensions to be irradiated by laser pulses. Subsequent photoacoustic waves are sensed by a transducer element. Here, water droplets are dyed with black ink to provide contrast.](image)

2.4 Two Phase Flow Generation

The consistency and reproducibility of two phase flow has been heavily investigated, especially for microfluidic systems. Two phase flow was created using a T-junction, which combines the two separate phases into one flow path while keeping the phases distinct, as shown in Fig. 2. The phases chosen for use in this system were air and water. Air and water were ultimately preferred because the desired water slug could easily be extracted without contaminating the sample with the neighboring phase. However, when using air and water, the liquids build up pressure and purge the system. Fortunately, successful use of surfactants, such as Tween 20, have been reported in an air/water two phase flow system. These surfactants reduce the interfacial tension between the phases and Tween 20 was ultimately used in our flow system. A 2% Tween 20 (Fisher Scientific, Pittsburgh, Pennsylvania) in PBS buffer solution (Fisher BioReagents, Pittsburgh, Pennsylvania) was used as the water phase. Figure 3 shows slug formation using flow rates of 100, 200, and 400 μL/min for air and 50 and 100 μL/min for water. The regular spacing was made possible by the addition of the surfactant. Without surfactant, overpressure and irregular spacing occurred.

The system reported is shown in Fig. 4 and is different from most other microfluidic systems due to its size and shape. The system is composed of cylindrical silicone tubing (Masterflex, platinum cured silicone tubing, LIS14, Cole Parmer, Vernon Hills, Illinois), which behaves slightly differently than square edge systems. Although a classic microfluidic system utilizes channel dimensions lower than 100 μm and flow rates less than 1 μL/s, our system used a tubing inner diameter of 1.6 mm and the flow rates used ranged from 100 to 200 μL/min. The slugs that formed were only 3 to 4 μL and the Capillary and Reynolds numbers stayed within microfluidic conditions. In addition, high flow rates were used to achieve large volume throughput.

2.5 Concentration Study

The concentration study photoacoustically evaluated melanoma concentrations of 5 cells/μL, 1 cell/μL, 1 cell/5 μL, 1 cell/10 μL, and 1 cell/25 μL suspended in PBS +2% Tween 20 with a control of PBS +2% Tween 20.

**Transducer**. A commercial Vivo 770 Imaging System (Visual Sonics, Inc., Toronto, Ontario, Canada) which contains an ultrasound probe (RMV-708) was used in the concentration study. The acoustic bandwidth was 25 to 75 MHz and a complete description of this instrumentation set-up is described in.

**Laser**. A frequency tripled, Q-switched Nd:YAG laser (Continuum Surelight I-20, Santa Clara, California) was fired from above the flow path of the flow chamber, directly toward the ultrasound probe. A 1 cm thick scattering pad was placed
between the flow chamber and transducer to prevent any laser light from directly hitting the ultrasound probe while allowing acoustic waves to penetrate through to the probe. The laser pulsed at 532 nm, 20 Hz, and between 3 and 6 mJ for a pulse duration of 5 ns.

Flow system. The flow chamber was connected to two syringe pumps; one syringe contained the cell samples and the other contained air. The air was pumped at 0.2 mL/min and the cell samples were pumped at 0.1 mL/min. The syringe pump that housed the cells was set vertically to ensure the cells did not settle to the bottom of the syringe, therefore the air needed a higher flow rate to compensate for the increased pressure.

2.6 Blind Study

A blind study was designed in which melanoma cells were spiked in PBS +2% Tween 20 with a concentration of 10 cells/μL and the photoacoustic flow cytometer was used to detect them. We randomly chose samples for melanoma spiking, the remainder of the samples served as controls. With a 50% chance of cell spiking, we had 13 samples spiked with melanoma and 7 without melanoma. The operator of the flow cytometer used the presence of photoacoustic waves to determine if a sample contained melanoma cells and classified all 20 samples correctly.

Transducer. A 25 μm thick polyvinylidene fluoride (PVDF) piezoelectric film (Piezotech S.A.S., Hensingue, France) in brass housing was used as the transducer. We did not complete bandwidth analysis but a central frequency of 45 MHz was calculated based on the thickness of the element and the speed of sound in PVDF. It was sandwiched between a Poly dimethylsiloxane (PDMS) base (Sylgard, Dow Coming Corporation, Midland, Michigan), and a 1.5 mm thick PDMS ring that covered the top of the brass housing, yet still revealing the PVDF element to prevent acoustic reflections. The transducer...
was fitted into a polyvinyl chloride holder that helps align the transducer, the PDMS components, and finally the flow chamber, as shown in Fig. 1.

**Laser.** The same laser used in Experimental Set-up 1 was fired at a right angle to the transducer, entering on the side of the flow chamber. The flow chamber was directly coupled to the transducer with ultrasound gel.

**Flow system.** The same system used in the concentration study was used in the blind study.

### 2.7 Capture and Imaging of Melanoma

The flow system was prepared using one syringe pump containing air, and one syringe pump containing cultured melanoma cells in PBS and 2% Tween 20. A PBS and air control was conducted to show that no photoacoustic signals were produced from either air or PBS slugs. Melanoma concentrations of 500, 400, 300, 200, 100, 50, 25, 10 and 1 cells/μL were directed through the system, and slugs that produced photoacoustic signals when passing through the detection volume were extracted from the system to be imaged. The collected samples were allocated to different glass slides based on concentration, and fixed to glass slides using a Cytospin 4 centrifuge (Thermoscientific, Kalamazoo, Michigan). After centrifugation, the slides were stained using a Fontana Masson staining kit and protocol (Shandon EZ Single Cytofunnels, Scytek Laboratories, West Logan, Utah) and finally mounted in a resin. The slides were imaged using wide field microscopes in the University of Missouri Molecular Cytology Core.

**Transducer.** The same transducer that was used in the blind study was also used in the melanoma capture and imaging studies.

**Laser.** The same laser set-up that was used in the blind study was also used in the melanoma capture and imaging studies.

**Flow system.** The same system used in the concentration study was used in the melanoma capture and imaging studies.

### 3 Results

#### 3.1 Photoacoustic Signals by Concentration

Photoacoustic waveforms from slugs filled with different concentrations of melanoma cells are shown in Fig. 5. The data was quantified as percentage of slugs that were predicted to be positive for melanoma and those that were negative as a function of concentration as shown in Table 1. As the concentration decreased, the predicted and actual percentages of slugs with events decreased mutually. The predicted percentage was calculated by assuming homogenous distribution of melanoma cells within the sample volume and then dividing the total number of cells in the sample by the slug volume of 3 to 4 μL.

#### 3.2 Blind Study

The results from the blind study are shown in Table 2. The sample number corresponds to the order in which the samples were tested, and the content refers to whether or not melanoma was present. M signifies that melanoma was present, and nm signifies that melanoma was not present. The last row displays the decision made by the blind study operators; + indicates that the researchers believed melanoma was present in the sample based on the photoacoustic response, and − indicates that the researchers believed melanoma was not present in the sample based on the response. The researchers classified all 20 samples correctly.

### 3.3 Capture and Imaging of Melanoma

The system produced no signals for the PBS baseline, and produced photoacoustic signals from each different concentration of melanoma slugs. Figure 6 on the right shows the photoacoustic response of 10 melanoma cells/μL compared to data taken from a sample of separated health blood shown on the left. Slugs that produced photoacoustic signals were captured and then stained using the Fontana Masson stain.

Slugs from the healthy blood sample were captured and imaged and a white blood cell from the sample is shown in Fig. 6(a) and 6(c). Slides that contained low concentrations of melanoma did not reveal obvious melanoma cells during imaging. This is likely due to the harsh staining procedure that requires the slides to be washed numerous times, causing many of the cells to wash off of the slide. When the melanoma concentration was higher (≥100 cells/μL), melanoma cells were easily found via imaging as shown in Fig. 6(b) and 6(d) due to the higher number of cells initially fixed to the slide. The Fontana Masson Stain turns the nucleus red and turns melanin black.

### 4 Discussion

Capture of rare cells flowing through a flow system or even directly from the circulatory system in vivo is extremely difficult because of inherent properties of laminar flow. It is impossible to predict with 100% certainty where a cell will be located because of drag forces that occur along the walls of the tubing or veins. This problem has been resolved by incorporating two phase flow into a flowmeter, which breaks down the fluid into alternating droplets of sample and droplets of air. The contents of each slug will stay within the same droplet for the entire duration of flow, and thus an analyte of interest can be tracked once the droplet that contains it has been identified.

While searching for the optimal phases to use for our studies, we found that successful slug formation was achieved using both oil/water and air/water and surfactant, but ultimately water and air with the addition of a surfactant were the choice fluids due to the ease with which melanoma cells can be extracted from the flow system. The addition of Tween 20 was effective due to its ability to decrease surface tension between the two phases allowing water to slide past the air when pressure builds up. The ratios of air droplet volume and sample droplet volume can be changed by altering the flow rates of the respective phases as seen in Fig. 3. This simple and inexpensive technique can easily isolate small volumes of a sample where an analyte of interest is located. Two phase flow isolation can be used with photoacoustic detection, but could also be helpful for captured analytes that are detected via fluorescence, flow cytometry, and other methods.

The concentration study validated the method since the predicted percentage and actual percentage of slugs that had photoacoustic effects were nearly identical showing high sensitivity as shown in Table 1. The control samples had zero false positive signals indicating that the specificity of the method is also very high. For the concentrations where the expected number of
melanoma cells per detection volume was less than 1, only some of the slugs produced photoacoustic signals as expected. The negative bubbles looked nearly identical to the PBS + Tween 20 control waveforms. More trials must be run to validate these statements through statistical analysis, but the preliminary results are very encouraging. The control photoacoustic signal was not completely baseline; however the waveform is an artifact whereas photoacoustic signals generated from melanoma are transient in nature, as expected from a chromophore that is not stationary with respect to the transducer. This phenomenon makes it effortless to decipher between noise and melanoma cells.

Usefulness of this method in a clinical setting was confirmed from the melanoma blind study, in which all 20 samples were
identified correctly. Furthermore, the melanoma cell capture and imaging proved that the system can effectively isolate detected melanoma cells from the system. This method is a significant step toward both an efficient disease monitoring technique and a diagnostic tool, and will also enable scientists to study these metastatic cells in hopes of discovering the mechanisms by which they metastasize. New knowledge could result in the production of better therapies for cancer patients, including the prevention of metastatic cancer. Characterization of CMCs will also allow for personalized therapy, increasing the number of successful patient treatments.

By staining the captured cells with the Fontana Masson Stain, we have confirmed that melanoma cells were truly detected, validating all of the work presented in this paper. Future work involves performing these tests with blood from metastatic melanoma patients, and eventually taking the system to clinical trials. For patient blood samples, the isolated samples will be stained using immunohistochemistry: a MART1

Table 1  The percentage of predicted and actual number of slugs with photoacoustic signals based on CMC concentration. The predicted percent of slugs with events decreases as concentration goes down, and the actual percentage followed this trend as well.

<table>
<thead>
<tr>
<th>CMC concentration</th>
<th>Slugs without events</th>
<th>Slugs with events</th>
<th>Total slugs</th>
<th>Predicted % of slugs with events</th>
<th>Actual % of slugs with events</th>
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<tbody>
<tr>
<td>Control</td>
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<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
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<tr>
<td>5 cells/µL</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1 cell/µL</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1 cell/5 µL</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>60 to 68%</td>
<td>70%</td>
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<tr>
<td>1 cell/10 µL</td>
<td>15</td>
<td>5</td>
<td>20</td>
<td>30 to 40%</td>
<td>25%</td>
</tr>
<tr>
<td>1 cell/25 µL</td>
<td>25</td>
<td>5</td>
<td>30</td>
<td>12 to 16%</td>
<td>16.70%</td>
</tr>
</tbody>
</table>

Table 2  Twenty samples were tested in a blind study. Here, content refers to presence of melanoma or not, where m indicates melanoma and nm indicates the absence of melanoma. For the test, + indicates the test showed presence of melanoma, while – indicates no melanoma detected.

<table>
<thead>
<tr>
<th>Sample</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Content</td>
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<td>m</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6 (a,c) Irradiating white blood cells yields no photoacoustic effect. (b,d) Irradiating melanoma cells among white blood cells yields photoacoustic waves. Capturing droplets containing the cell suspensions that generate photoacoustics waves shows the presence of pigmented melanoma cells.
stain for melanoma cells, a CD-45 stain for leukocytes, and an EpCAM stain for cells of epithelial origin, which are otherwise absent in blood cells. Isolated samples should be positive for the MART1 stain and positive for the EpCAM stain while the CD-45 stain will be used as a leukocyte control.

Expansion of this technique to other cancers is being pursued using various chromophores including gold nanoparticles and pigmented microspheres. Our group has successfully detected prostate cancer cells by tagging them with gold nanoparticles, and is currently pursuing ways to detect breast cancer using the HER-2 antibody with both nanoparticles and microspheres. These are significant steps toward a photoacoustic detection and isolation system that can be used for all types of cancer.

One of the greatest advantages this technique has over other methods being investigated is the ease of use and small cost per test. The system is currently being automated to both detect and isolate single CTCs with a simple push of a button. First, system will direct the purified and compartmentalized blood through the system. Next, positive slugs will be classified by an amplitude threshold and will be shunted off into a special compartment, while negative slugs are purged from the system. The positive slugs will flow back in the opposite direction and be diluted inline. The diluted solution will be compartmentalized with another T-junction and passed through the detection volume. Positive slugs will again be shunted off into a special compartment, and negative slugs will be purged from the system. This vacuuming process will continue until only melanoma cells exist in suspension with PBS. After loading the separated blood sample into a syringe, the entire process will be automated using LabView, with the end result of a small volume of melanoma cells residing in PBS.

For the experiments presented in this paper, the sample flow rate was set to 0.1 mL/min. This rate was chosen so that the oscilloscope could average the signals 16 times in order to improve the signal to noise ratio (SNR). However, averaging is not necessary if the SNR is acceptable from single laser pulses which can be achieved by various methods such as using a more sensitive transducer. This would allow the flow rate to be restricted only to the frequency of the laser pulse and staying within microfluidic conditions for generation of two phase flow. The resulting higher flow rates could allow the test to be completed in less than 5 min.

The one time, fixed cost of purchasing a laser and other instrumentation are the only large costs, whereas the test will only include the cost of syringes and tubing. These qualities make it a prime technology for clinical use, and if we can increase our analyte detection to all types of cancer, this method may have an edge over existing and currently developing technologies.

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