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# Cell streak imaging cytometry for rare cell detection

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ABSTRACT

Detection of rare cells, such as circulating tumor cells, have many clinical applications. To measure rare cells with increased sensitivity and improved data managements, we developed an imaging flow cytometer with a streak imaging mode capability. The new streak mode imaging mode utilizes low speed video to capture moving fluorescently labeled cells in a flow cell. Each moving cell is imaged on multiple pixels on each frame, where the cell path is marked as a streak line proportional to the length of the exposure. Finding rare cells (e.g., $<1$ cell/mL) requires measuring larger sample volumes to achieve higher sensitivity, therefore we combined streak mode imaging with a "wide" high throughput flow cell (e.g. flow rates set to 10 mL/min) in contrast to the conventional "narrow" hydrodynamic focusing cells typically used in cytometry that are inherently limited to low flow rates. The new flow cell is capable of analyzing 20 mL/min of fluorescently labeled cells. To further increase sensitivity, the signal to noise ratio of the images was also enhanced by combining three imaging methods: (1) background subtraction, (2) pixel binning, and (3) CMOS color channel selection.

The streaking mode cytometer has been used for the analysis of SYTO-9 labeled THP-1 human monocytes in buffer and in blood. Samples of cells at 1 cell/mL and 0.1 cell/mL were analyzed in 30 mL with flow rates set to 10 mL/min and frame rates of 4 fps (frame per second). For the target of 1 cell/mL, an average concentration of 0.91 cell/mL was measured by cytometry, with a standard error of 0.03 ($C_{95}$ $=0.85–0.97$). For the target of 0.1 cell/mL, an average concentration of 0.083 cell/mL was measured, with a standard error of 0.01 ($C_{95}=0.065–0.102$). Whole blood was also spiked with SYTO-9 labeled cells to a concentration of 10 cell/mL, and the average flow cytometry measurement was 8.7 cells/mL (i.e. 0.87 cells/mL in diluted blood) with a 95% CL of 8.1–9.2 cells/mL. This demonstrated the ability to detect rare cells in blood with high accuracy. Such detection approaches for rare cells have many potential clinical applications. Furthermore, the simplicity and low cost of this device may enable expansion of cell-based clinical diagnostics, especially in resource-poor settings.

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## 1. Introduction

Rare cells, including circulating tumor cells (CTCs), have many promising clinical applications. Current flow cytometry techniques commonly used for cell analysis are not well suited for rare cells analysis where large sample volumes is needed. The reason for this is they utilize hydrodynamic flow focusing to constrain the spatial location of cells to a very narrow region so that cells can be interrogated individually by highly sensitive optical point or line detectors (e.g., photomultiplier tubes). This approach ultimately limits the device to small volumes. Analysis of large volumes requires redesign of the flow cytometer flow cell to interrogate wider flow fields. To address these issues, full-field imaging sensors was used for optical detection combined with classical flow cytometry (Zuba-Surma and Ratajczak, 2011). However, high throughput flow cytometers will require both high flow rate cells and the adaptation of wide-field imaging detectors for the analysis of such flow cells.

Because of the inherent wide-field capabilities of imaging sensors, CMOS or CCD cameras have been employed in several array assays (Ligler et al., 2007; Moreno-Bondi et al., 2006; Ngundi et al., 2006; Taitt et al., 2005). Their main advantage is they can be used for analyzing light from a large enough area that it can cover the entire surface of a lab-on-a-chip (LOC) or an array (Kostov et al., 2009; Sapsford et al., 2008; Sun et al., 2010). Recently, optofluidic fluorescent imaging cytometry on a cell phone with a spatial resolution of ~2 μm was described (Zhu et al., 2011; Zhu and Ozcan, 2013). While very mobile and versatile, the flow rate of this system is ~1 μL/min, which limits analysis to small volumes.

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To adapt flow cytometry analysis to the larger sample volumes that are needed for rare cell analysis, we designed a high throughput wide ("2D") flow cell that combined a wide-field low cost webcam for cell imaging (Balsam et al., 2014). To increase the sensitivity of this device for rare cell detection, we report here a new cell streak imaging mode that captures moving fluorescently labeled cells in a flow cell at low video imaging speeds. This technique reduces the amount of data needed for analysis, making it more suitable for use with low sensitivity and high noise webcams or mobile phone cameras being used in cell-based clinical diagnostics for mHealth, especially in resource-poor settings.

## 2. Materials and methods

### 2.1. Flow-cell fabrication

A wide flow cell (Fig. 1B) was fabricated using an Epilog Legend $CO_2$ 65 W laser cutter (Epilog, Golden, CO) as described in our previous work (Balsam et al., 2014). The flow cell consisted of three functional layers: (1) a plain glass or quartz microscope slide lower layer, (2) a middle layer laser machined from 3M 9770 double-sided adhesive transfer tape to define the geometry of the fluid channel, and (3) a glass or quartz microscope slide on the top layer that has two holes drilled for the inlet and outlet ports aligned with the ends of the fluid channel layer.

### 2.2. Webcam-based flow cytometer

As describe in previous work (Balsam et al., 2014), a Sony PlayStation® Eye webcam or Point Grey Research equipped with a c-mount CCTV lens (Pentax 12 mm f/1.2) were used as the photodetector. For florescence detection, a green emission filter with center wavelength 535 nm and bandwidth 50 nm (Chroma Technology Corp., Rockingham, VT) was used for detecting fluorescent emission. For fluorescent excitation, a 1 W 450 nm laser was used (Hangzhou BrandNew Technology Co., Zhejiang, China). Camera control software for webcams (CL-Eye Test) was used to set the camera parameters (exposure time, frame rate, and gain) and to capture and save video in uncompressed AVI format. Video files were analyzed using ImageJ software (http://rsb.info.nih.gov/ij/download.htm).

### 2.3. Cell SYTO-9 labeling dilution

To simulate rare cells, fluorescently stained THP-1 human monocytes were used. Cells were centrifuged and resuspended in PBS. 10 μL SYTO-9 dye (5 mM stock concentration) was added to 1 mL of suspended cells and allowed to rest at room temperature in the dark for 20 min. Cells were then pelleted and washed three times with PBS to remove excess dye. After labeling, cells were diluted to a level of approximately 1 cell/μL (measured by microscopy) to allow for manual counting. Human whole blood (single draw, O+) was purchased from BioChemed Services (Winchester, VA). 3 mL samples of whole blood was diluted 10× in 25 mM HEPES buffer for a final volume of 30 mL. THP-1 monocytes were resuspended in 25 mM HEPES. 10 μL of 5 mM SYTO-9 nucleic acid fluorescent stain (Life Technologies) was added to 1 mL of THP-1 and the cells were spiked into to each 30 mL diluted blood sample to yield a final concentration of 100 cells/mL of whole blood.

## 3. Results and discussion

To improve sensitivity and to better utilize the 2D imaging capability of the webcam for rare cell detection, we developed streak mode detection to image the cells on multiple pixels as lines and not as individual points as in conventional imaging.

### 3.1. High volume flow cytometer for rare cell detection

The imaging flow cytometer (Balsam et al., 2014) consists of four modules (Fig. 1A): (1) a webcam utilized as imaging sensor, (2) a blue 450 nm 1 W laser excitation source, (3) a high throughput flow-cell, and (4) a focusing stage for image focusing and alignment. The sensor includes the internal electronics of the webcam, a 12 mm f/1.2 CCTV lens and two green emission filters. The webcam was connected to a computer which was used to power the webcam and to collect and analyze data. The fluid handling system include the flow-cell (Fig. 1B) and a programmable syringe pump.

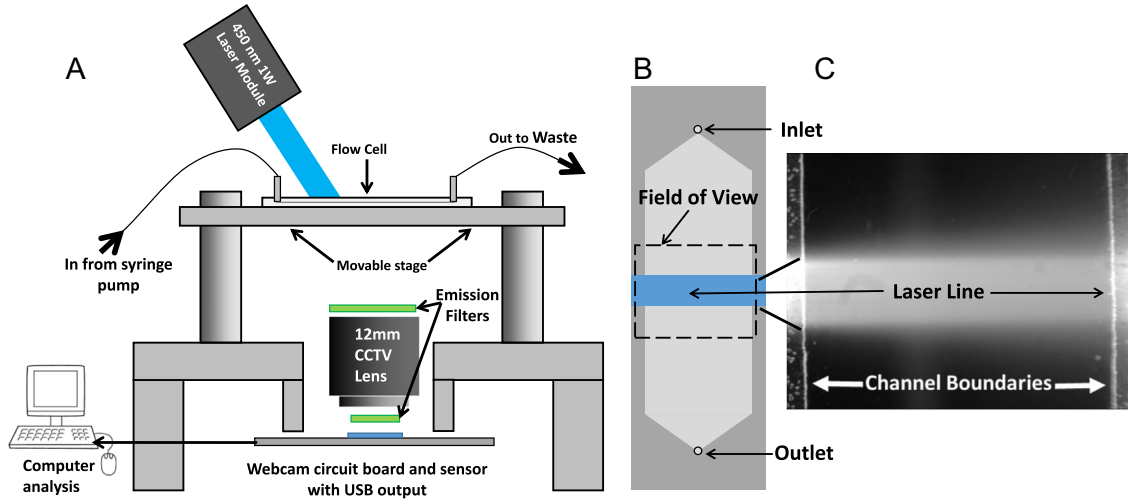


**Fig. 1.** Schematic of webcam-based wide-field flow cytometer – (A) The flow cytometer consists of four modules: a sensing element, excitation source, flow cell, and a stage to hold each module in alignment. The sensing element consists of the internal elements of a webcam, a 12 mm f/1.2 CCTV lens, two green emission filters, and a computer to collect and analyze data. The excitation source is a 450 nm 1 W laser module. The sample handling module consists of a flow cell and a programmable syringe pump. (B) A schematic of the wide-field flow cell is shown with camera field of view and excitation laser line indicated, along with (C) an image from the camera showing the same features. The laser line is visible in this image due to autofluorescence of the glass flow cell.