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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 ($C_{95} = 0.85\text{--}0.97$). 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 ($C_{95} = 0.065\text{--}0.102$). 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.

Keywords

Flow cytometry; Wide-field imaging; Rare cells; Resource-poor settings; Image enhancement; Background subtraction; Pixel binning; mHealth

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 $\sim 2 \mu\text{m}$ was described (Zhu et al., 2011; Zhu and Ozcan, 2013). While very mobile and versatile, the flow rate of this system is $\sim 1 \mu\text{L}/\text{min}$, which limits analysis to small volumes. 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 CO₂ 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 μ L 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/ μ L (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 $\times$ in 25 mM HEPES buffer for a final volume of 30 mL. THP-1 monocytes were resuspended in 25 mM HEPES. 10 μ L 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.

In order to maximize residence time of cells in the interrogation the flow channel was widened to 20 mm. Channel depth was not increased in order to keep the flow field within the depth of field of the lens. In order to provide approximately uniform excitation across the width of the channel, the laser source was injected into the side of the flow cell such that it formed a linear band of excitation across the center of the field of view, as seen in Fig. 1C.

The distance between the imaging lens and the flow cell was set such that images of cells were projected onto at most a 3×3 array of pixels. When cells are imaged on more than one pixel, photon flux per pixel is a constant and independent of the distance between flow cell and lens. The largest possible distance should be chosen in order to maximize field of view (FOV). In the setup used here the lens was placed at a distance of approximately 20 mm from the webcam CMOS enabling the lens to focus at very close range on the entire detection field of the flow cell.

3.2. Cell streak imaging

One problem which was encountered with high speed imaging for the application of cell counting was that cells were captured as spots on small number of pixels (which look similar to spots of noise in an image) and the high frame rates required resulted in video files which were excessively large. At a flow rate of 500 $\mu\text{L}/\text{min}$, a frame rate of 187 fps (the camera maximum) was required to avoid motion blur. At that rate, analysis of a 7.5 mL sample would generate a 50 GB video file comprised of 170,000 individual frames in 15 min. Analysis of files of that size is cumbersome and time consuming, and storage of many sample files quickly becomes difficult for personal computers and impossible for phone based detection.

By increasing the flow cell volume by a factor of 5–2500 $\mu\text{L}/\text{min}$, flow rates could be increased by a similar factor to reduce sample time to 3 min and file sizes to 10 GB each. However, this would still become unmanageable quickly on portable diagnostic platforms, such as cell phones, when performing dozens of tests per day. To solve this problem we adopted a technique from fluid velocimetry known as streak photography which allowed for the measurement of fluid velocities with the low frame rate film cameras in a streak imaging mode. Exposure times and fluid velocities are set such that the illuminated particles formed short streaks in the final images. The direction and relative length of these streaks can be used to measure localized fluid motion (e.g., velocity, vorticity, etc.). Here, we use cell streak imaging to improve SNR of images of fluorescently labeled cells in order to improve their detection and to reduce data requirements. Frame rates are reduced to the range of 4–10 fps, which also reduces the data storage requirements by a factor of 20–40. Cell image SNR is also improved, as discussed next.

When imaging a stationary fluorescent cell, cell image brightness increases with exposure time. When imaging a moving fluorescent cell, cell image brightness increases with exposure time up to a point and then remains essentially constant for increasing exposure times. This transition point is the exposure time that allows the cell image to traverse a distance equal to its length in pixels plus one pixel (where the pixel in the middle of the cell image streak will have the maximum brightness). A longer exposure time will produce a longer streak of pixels with a brightness that is equal to this maximum value (plus noise components). This is shown graphically in Fig. 2A, where a cell of length 3 pixels is seen to move a distance of 4 pixels, thereby producing an image streak with a single bright pixel in its center. An actual cell streak image is shown for a similar cell size and similar flow conditions, along with a plot of pixel brightness along the center of the cell streak.

As depicted in Fig. 2B, for a given exposure time and flow rate, a cell will move a given distance and have a characteristic image brightness (Fig. 2B-i). If exposure time is increased (Fig. 2B-ii), streak length will increase and average streak brightness will remain constant. If flow rate is increased (Fig. 2B-iii) streak length will increase and average streak brightness will decrease.

3.3. Fluorescent signal analysis of streak mode images

An actual cell streak image (circled) with flow direction indicated is shown in Fig. 2C with a close-up of the cell streak image showing individual pixels and background noise (Fig. 2D). In order to reduce noise, each column of pixels is averaged along the streak length n to produce a single averaged row of pixels, labeled $\text{avg}(n)$ in Fig. 2D. A plot of pixel values before and after averaging are shown (Fig. 2E-i and ii, respectively), indicating a $3 \times$ improvement in SNR. SNR was calculated as

$$SNR = \frac{\mu_{\text{signal}}}{\sigma_{\text{noise}}}$$

where μ_{signal} is the peak value in the plot, and σ_{noise} is the standard deviation of the pixel values on either side of the peak. When calculating σ_{noise} , a five pixel exclusion zone on either side of a peak was used to prevent the inclusion of any signal components in the noise measurement. Twenty-five pixel windows on either side of this exclusion zone were used to measure local noise levels (i.e. a total of 50 pixels around each peak).

3.4. Streak imaging signal enhancement

One of the challenges in fluorescence imaging is noise. We combined three approaches to increase signal to noise ratio: (a) image background subtraction, (b) pixel binning, and (c) color channel extraction.

- a. *Background subtraction.* Image subtraction was used to remove the constant components of background noise and improve local signal to noise ratio. Fig. 3A shows a raw video frame with two faint fluorescent cell streaks (circled and marked with arrows) and the bright horizontal band of excitation laser line auto-fluorescence at the center. To improve cell streak visualization, the average background signal of a data set was subtracted from each constituent frame. Fig. 3B shows the average value of 720 video frames from one sample. This average video frame was subtracted from each individual frame in the data set, and its qualitative effect on the frame shown in Fig. 3A is shown in Fig. 3C, where the cell streaks are more readily distinguished. Averaging was carried out using the `zProject` function in ImageJ. The result is a single image which is the average of all video frames. Such an image necessarily contains contributions from the fluorescent cell streaks. However for rare cell counting such events do not significantly affect the subtraction process. A more sophisticated averaging algorithm could be used to remove the contribution of bright cell streaks if it was found that they substantially influenced the background levels.

- b. Pixel binning.** Pixel binning was used to reduce the effects of random noise sources. The full resolution video frames (640×480 pixels) from a 30 mL sample (1 cell/mL) were binned 1×480 in post-processing such that each frame had a final resolution of 640×1 (i.e. each column of pixels was averaged to produce a single pixel). Fig. 4A is a single video frame prior to binning, showing a single vertical cell streak. The direction of binning is shown with an arrow. The one dimensional intensity measurement of the horizontal line shown in this figure was analyzed (Fig. 4B), showing the maximum single pixel value of the cell streak along with the surrounding noise. The SNR of the peak value is 5. The one dimensional intensity measurement of the video frame shown in Fig. 4A after being binned in the vertical direction is shown in Fig. 4C. The SNR of the peak value is 15, improving SNR by a factor of three. To count all the labeled cells, all 720 frames from a 3 min 4 fps video file presented as a single image (Fig. 4D), where each horizontal line is one binned frame. Vertical position of cells (circled) indicates relative arrival time. The 3D representation of Fig. 4D is shown in Fig. 4E. The “Orthogonal Views” function in ImageJ was used for this purpose.
- c. Color channel extraction.** The webcam CMOS sensor employs a typical Bayer color filter array, in which one half of all pixels have a green filter, 25% have a red filter and 25% have a blue filter (Fig. 4F). The emission profile of SYTO-9 dye used in this work is mainly in the green spectrum (Fig. 4F) with the emission spectrum of SYTO-9 overlaid showing the overlap with the three color channels. The large overlap of this emission profile with the green spectrum results in this color channel having the highest SNR. The blue and red channels typically showed SNRs of one half and one tenth those of the green channel. For this reason the red and blue channels were discarded and only the green channel was analyzed. The “Split Channels” function in ImageJ is used to divide a color image into its constituent channels.

3.5. Cell velocity in streak imaging cytometry

The flow rate is a major factor of analysis of large sample volume needed for detection of rare cells. To study the relationship between volumetric sample flow rate and linear particle velocity in the wide-field flow cell field of view must be established. Labeled THP-1 monocytes were injected at flow rates between $100 \mu\text{L}/\text{min}$ and $20 \text{ mL}/\text{min}$. The cells (marked with arrows in Fig. 5) were captured at 20 fps (exposure time $1/20 \text{ s}$). The length of the streak is proportional to the flow rate (Fig. 5A–D). At 20 fps, cells flowing at $100 \mu\text{L}/\text{min}$ (Fig. 5A) are shown as individual points while at $20 \text{ mL}/\text{min}$ the cells are shown as a long streak. Streak lengths were measured and divided by exposure time to determine velocity in units of pixels/s. It was found that there were distinct linear (Fig. 5E) and non-linear ranges of operation (Fig. 5F). Flow rate and cell velocity were linearly correlated below $1000 \mu\text{L}/\text{min}$. Above this speed, the relationship became nonlinear. Non-linearity in the relationship between flow rate and particle velocity can be attributed to viscoelastic creep in the double-sided adhesive transfer tape used to fabricate the flow cell, resulting in increasing cross-sectional area.

3.6. Counting efficiency of cell streak imaging cytometry

To determine the counting efficiency of the described cell streak cytometer, sample of human monocytes cells were counted and compared to counts obtained manually via fluorescent microscopy as described in Section 3.5. Manual counting was performed at a higher stock concentration (1000 cells/mL) and the results for each target concentration were calculated following serial dilution. Samples of cells at 1 cell/mL and 0.1 cell/mL concentrations were analyzed in batch sizes of 30 mL with flow rate set to 10 mL/min and frame rate 4 fps. Fig. 6A shows the results of these experiments with the measured stock concentrations (sparsely filled columns) and the cytometer results (densely filled columns) for the average concentration measured over all samples, the error bars represent a 95% confidence interval for each data set. 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 ($C_{95} = 0.85\text{--}0.97$). 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 ($C_{95} = 0.065\text{--}0.102$).

Fig. 6B and C show distributions of measured cell concentrations per sample for the both 1 and 0.1 cell/mL cases. For each data set a Poisson distribution is plotted with λ set equal to the target mean concentration. Both cases show reasonably good fits with theory. In the experiments for 1 cell/mL, a total of 27 samples were taken 30 mL at a time and yielded an average cell count of 27.3 cells/sample. A total of 737 cells were measured in this data set. The probability density function of a Poisson distribution with λ equal to the predicted population average of 30 cells/sample is shown (dotted line). For the 0.1 cell/mL data set, a total of 20 samples were taken 30 mL at a time and yielded an average cell count of 2.5 cells/sample. A total of 50 cells were measured in this data set. These data suggest that in buffer the flow cytometer is capable of reliable detection of 0.1 cells/mL.

3.7. Cell streak imaging of labeled THP-1 human monocytes in blood

To determine the counting efficiency of cell streak imaging of SYTO-9 labeled cells in a complex biological background, labeled cells were spiked into whole blood in a concentration of 10 cells/mL. Because cell lysis may lead to loss of some of the few available rare cells, we simply diluted the blood by 10X in HEPES buffer. This yielded a final concentration of 1 cell/mL as measured.

Twenty-three 3 mL samples of whole blood were spiked to a target concentration of 10 cell/mL, followed by dilution into 27 mL HEPES. A flow rate of 500 $\mu\text{L}/\text{min}$ and frame rate of 10 fps were used. As shown in Fig. 6D, the average flow cell concentration measured by flow cytometry was 8.7 cells/mL (i.e. 0.87 cells/mL in the diluted state), with a 95% CL of 8.1–9.2 cells/mL. The measured spiked cell concentration in whole blood was 13% less than the calculated expected concentration.

The primary factor that resulted in this undercounting is probably the absorption of the blue excitation light by the RBCs. It was noted that cells closest to the injection point of the laser tended to show brighter emission than those farthest from the excitation source. Fig. 6E shows a typical cell passing close to the laser injection point where intensity is highest, and Fig. 6F shows a typical cell passing through the region farthest from the injection point

(where intensity is lowest) which is almost indistinguishable from background. Removal of the RBC by centrifugation may overcome this problem. This also suggest that illumination from both side of the flow cell may improve illumination and cell detection.

One potential limitation with cell streak imaging cytometry is two cells that are overlapped or adhered together will be counted as a single cell. However, the method is designed for detecting rare cells (e.g. 1 cell per 10 mL) and the probability that two rare cells will appear in a single frame at the same position is extremely low. Furthermore, if by change two cells were to appear in the same frame, the probably of overlap is on the order of 10^{-9} , which means that overlapped or adhered cells will not be a significant contributor to error in rare cell detection.

4. Conclusions

A wide-field cytometer is presented here that is capable of high throughput (flow rates of 10 mL/min) flow analysis of cells, in contrast to the conventional low flow rates associated with hydrodynamic focusing flow cell configurations typically used in cytometry. Combined with wide-field video imaging utilizing a low cost webcam and streak imaging mode, this approach enables detection of low cell concentrations (e.g., 1 cell per 10 mL) at high throughput. By using streak imaging, the data requirements for a test are reduced by an order of magnitude, while detection sensitivity can be enhanced for faint signals. This wide-field video imaging cytometer device demonstrates the capability of streak mode imaging to count rare cells in blood with a high level of accuracy. This level of detection is at a clinically relevant range for rare cells, such as CTCs, and the high flow rate enables more rapid detection than conventional flow cytometers. Wide-field flow cytometry combined with cell streak imaging results in a simpler, cheaper, and more portable flow cytometer, which facilitates the expansion of cell-based clinical diagnostics, especially in resource-poor settings.

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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.

Fig. 2.

Streak mode imaging principles – (A) Schematic of a cell (i) traversing a number of pixels equal to its length plus one pixel, showing (ii) a maximum brightness achieved in the pixel at the image center. (iii) An actual cell image is shown under these approximate flow conditions, along with (iv) a plot of its brightness along the center line of pixels. (B) An image with exposure time t will produce an image similar to (i), and exposure time $>t$ will produce an image similar to (ii), a longer streak with similar brightness. If exposure time is kept constant and flow rate is increased, a streak similar to (iii) will be produced: longer but darker. (C) A cell streak image (circled) with flow direction indicated. (D) Close-up of cell streak image showing individual pixels and background noise. In order to reduce noise, each column of pixels is averaged over the streak length n to produce a single averaged row of pixels, labeled $\text{avg}(n)$. (E) A plot of pixel values before (i) and after (ii) averaging, showing a $3 \times$ improvement in SNR. The plot in (i) is for the row with the brightest pixel value in (D).

Fig. 3.

Streak imaging signal enhancement – Green channel video images of samples passing through the flow cell were enhanced to improve cell image visibility. (A) Single raw webcam image of human THP-1 monocytes stained with SYTO-9 dye showing a fluorescent cell streak (circled and marked with arrows) with the excitation laser line autofluorescence at the center. The average of all 720 video frames from one sample yields (B) a single frame containing only background autofluorescent signal of the green channel of video. The background (B) is subtracted from (A) to yield (C) a final image with improved cell streak visibility.

Fig. 4.

Sample Processing and Image SNR – The full resolution video frames (640×480 pixels) from a 30 mL sample (1 cell/mL) were binned 1×480 in post-processing such that each frame had a final resolution of 640×1 . (A) A single video frame prior to binning, showing a single vertical cell streak. The direction of binning is shown with an arrow. (B) 1-D intensity measurement of the horizontal line in (A), showing the maximum single pixel value of the cell streak along with the surrounding noise. The SNR of the peak value is 5. (C) 1-D intensity measurement of the video frame shown in (A) after being binned in the vertical direction. The SNR of the peak value is 15. (D) All 720 frames from a 3 min 4 fps video file presented as a single image, where each horizontal line is one binned frame. Vertical position of cells (circled) indicates relative arrival time. (E) 3D representation of (D). (F) Typical spectra of RGB Bayer filter array from a CMOS image sensor, with the emission spectrum of SYTO-9 overlaid showing the overlap with the three color channels. The large overlap with the green channel demonstrates why the SNR in this channel is highest. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Fig. 5.

Streak Image Characteristics – (A)–(D) Four background-subtracted images of THP-1 monocytes (location and length indicated with arrows) captured at 20 fps are shown with varying flow rates. (E) The relationship between average cell velocity and flow rate in the linear range of flow cell operation, and (F) in the non-linear range of operation, with linear trend line plotted for comparison. Non-linearity in the relationship between flow rate and particle velocity is attributed to viscoelastic creep of the flow cell resulting in increasing cross-sectional area at higher pressures.

Fig. 6.

Webcam-based wide-field flow cytometer counting efficiency – (A) Results of two concentrations of human monocytes counted via microscopy (sparse fill) and using the optimized webcam-based flow cytometer operating in streak mode (dense fill). Error bars represent a 95% confidence interval for each data set. Manual counting was performed at a higher stock concentration (1000 cells/mL) and the results for each target concentration were calculated following serial dilution. (B) Histogram (bin size= 2) of cell counts for a 1 cell/mL stock concentration of THP-1 monocytes measured using streak-mode imaging. A total of 27 samples were taken 30 mL at a time and yielded an average cell count of 27.3 cells/sample. A total of 737 cells were measured in this data set. The probability density function of a Poisson distribution with λ equal to the predicted population average of 30 cells/sample is shown (dotted line). (C) Histogram of cell counts for a 0.1 cell/mL stock concentration with model Poisson distribution ($\lambda = 3$ cells/sample). A total of 20 samples were taken 30 mL at a time and yielded an average cell count of 0.83 cells/sample. A total of 50 cells were measured in this data set. Streak mode imaging results for fluorescently labeled THP-1 cells spiked into whole blood at a concentration of 10 cells/mL are shown. After spiking, samples were diluted $10 \times$ in HEPES buffer. (D) A histogram shows the result of measuring 23 samples (average pre-dilution concentration: 8.7 cells/mL, 95% CL: 8.1–9.2 cells/mL). (E) shows a single bright cell streak closest to the laser source where intensity is highest, and (F) shows a single faint cell streak furthest from the laser source where intensity is lowest.