High-throughput single-microparticle imaging flow analyzer

Keisuke Goda, Ali Ayazi, Daniel R. Gossett, Jagannath Sadasivam, Cejo K. Lonappan, Elodie Sollier, Ali M. Fard, Soo jung Claire Hup, Jost Adam, Coleman Murray, Chao Wang, Nora Brack, Dino Di Carlo, and Bahram Jalali

Optical microscopy is one of the most widely used diagnostic methods in scientific, industrial, and biomedical applications. However, while useful for detailed examination of a small number (≤10,000) of microscopic entities, conventional optical microscopy is incapable of statistically relevant screening of large populations (>100,000,000) with high precision due to its low throughput and limited digital memory size. We present an automated flow-through single-particle optical microscope that overcomes this limitation by performing sensitive blur-free image acquisition and nonstop real-time image-recording and classification of microparticles during high-speed flow. This is made possible by integrating ultrafast optical imaging technology, self-focusing microfluidic technology, optoelectronic communication technology, and information technology. To show the system's utility, we demonstrate high-throughput image-based screening of budding yeast and rare breast cancer cells in blood with an unprecedented throughput of 100,000 particles/s and a record false positive rate of one in a million.

Optical microscopy is one of the most widely used methods in a diverse range of advanced research, industrial, and clinical settings, including microelectronics, food science, oceanography, archaeology, environmental science, energy science, microbiology, mineralogy, and pathology (1–3). High information content spatial metrics provided by imaging are used to characterize microscopic particles such as emulsions, microorganisms, and cells in particle synthesis, ecosystem monitoring, biofuel formulation, drug discovery, histopathology, and cytology-based diagnostics (3–5). An impressive arsenal of optical microscopy methods enables imaging of cellular structures beyond the diffraction limit of light (6–8). Unfortunately, while there has been breathtaking progress in improving spatial resolution in the last two decades, mostly overlooked is the temporal resolution of imaging systems.

While useful for visual inspection of many individual microparticles without human intervention, conventional automated microscopy does not have high throughput and is hence unable to evaluate, analyze, and screen large populations with high statistical accuracy. High-throughput detection is essential to rapidly assay morphological and biochemical properties in a reasonable period of time. Unfortunately, the throughput of state-of-the-art automated microscopes is only up to approximately 1,000 particles/s (9–12), limiting the particle count. As a benchmark, this throughput is significantly lower than that of single-pixel high-throughput flow analyzers such as electronic particle counters (13, 14) and flow cytometers (approximately 100,000 particles/s) (15, 16), which trade increased throughput for a lack of spatial resolution. Because of this trade off, such systems are often unable to resolve single, multiple, and clustered particles or unusually shaped particles as well as to distinguish debris and nonspecific labeling and hence produce a large number of false positive events or inaccurate subpopulation counts.

While high-end digital cameras [i.e., charge-coupled device (CCD) and complementary metal-oxide-semiconductor (CMOS) cameras] are now able to perform imaging at a speed approaching 1 million frames/s (17–19), they are not well suited for high-throughput microscopy. First, a critical limitation that hampers imaging of particles flowing at high speeds is the relatively long shutter speed or exposure time (≥1 μs) (17–19) that results in motion blur and loss of resolution during the high-speed flow, hence degrading specificity. Another limitation is the time needed to download the image from the array of thousands of pixels. To achieve high frame rates, the number of pixels that are employed must be reduced in a process known as partial readout (17–21). The penalty is that image resolution is lost at high frame rates. Furthermore, there is a fundamental trade off between sensitivity and speed—at high frame rates, fewer photons are captured during each frame, leading to reduction in sensitivity. Finally, currently digital image processing cannot be performed in real time due to the storage and access of the massive amount of digital data that is produced during high-speed imaging and hence requires many days of off-line digital processing. This translates into a limited particle count, thus compromising statistical precision in high-throughput screening. These limitations prevent automated microscopy from analyzing a large population of particles with high statistical precision in a practical period of time.

To address the needs and fill in the technological gap between the automated microscope and high-throughput flow analyzer, we propose and demonstrate an automated flow-through single-particle optical microscopy system that can evaluate, analyze, and screen a large population of particles with high specificity, high sensitivity, and high statistical precision in a short time. This method builds on a unique integration of (i) an ultrafast optical imaging modality known as serial time-encoded amplified microscopy (STEAM) (22, 23) for blur-free imaging of particles in high-speed flow, (ii) inertial microfluidic technology for shear-free focusing and ordering of particles with inertial forces (24, 25), and (iii) hybrid optoelectronic image processing circuitry for


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

1To whom correspondence should be addressed. E-mail: goda@ee.ucla.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1204718109/-/DCSupplemental.
real-time image processing. The integrated system transforms particles in well-controlled microfluidic flow into a series of E-slides—an electronic version of glass slides—on which particles of interest are digitally analyzed. With the power of optoelectronic communication and information technologies, this property enables fully automated real-time image-recording and classification of a large number of particles through their morphological and biochemical features. To demonstrate our technology’s utility, we show real-time nonstop image-based identification and screening of budding yeast cells at different budding stages and rare breast cancer cells in blood with an unprecedented throughput of 100,000 particles/s and a record false positive rate of one in a million.

Results

Principle of the High-Throughput Imaging Flow Analyzer. Our system, which we refer to as the STEAM flow analyzer (Fig. 1A and SI Text and Table S1), consists of three subsystems: (i) the microfluidic device (Fig. 1B and SI Text and Table S2), (ii) the STEAM camera (Fig. 1A and SI Text and Table S1), and (iii) the real-time optoelectronic time-stretch image processor (Fig. 1C and SI Text and Table S3). An animated video (Movie S1) shows the integrated functionality of the system. The STEAM flow analyzer operates in three steps. First, particles are controlled to flow at a uniform velocity and focused and ordered by inertial lift forces in the microfluidic channel. Second, the STEAM camera takes images of the fast-flowing particles. Finally, the real-time optoelectronic time-stretch image processor processes the images optically and electronically and then performs automated particle screening in real time.

The STEAM flow analyzer employs inertial microfluidic technology (Fig. 1B and Movie S1). This method provides uniform positions and velocities for particles through intrinsic inertial lift forces (important for generation of clear E-slides) while eliminating the need for sheath fluid, which is traditionally required for hydrodynamic focusing in conventional flow cytometers. While inertial forces are typically assumed to be negligible in microfluidic systems, they can, in fact, be important when the flow rate in microchannels is high. Particles in finite-inertia confined channel flows focus to cross-sectional dynamic equilibrium positions that correspond with the fold of symmetry of the channel cross-section (26). Furthermore, longitudinal ordering (i.e., stabilized interparticle or intercell spacing along the length of the channel) results from inertial lift forces and the flow field acting together (27). In some of our previous work, we determined an optimal balance between inertial lift forces and Dean drag forces, counter-rotating vortices perpendicular to primary channel flow, to accurately position a range of particle sizes to a single streak with two heights within the channel at a high flow rate (26). Here we reduced the length of one of these optimized channels to decrease its pressure and lowered the height of the channel to bring the distinct equilibrium positions in the vertical plane into closer proximity. To ensure stability in real-time image processing, the microfluidic device was fabricated by replica molding in thermoplastic polyester due to its high stiffness and ability to sustain high pressures (SI Text, Fig. S1, and Table S2) (28).

The STEAM camera first captures fast sequential images with laser pulses and then stretches time-encoded images in time so that they can be digitized and processed in real time (see SI Text). During the time-stretch process, images are also optically amplified to overcome the thermal noise inherent in photon-to-electron conversion. In the first step, a pair of diffraction gratings spatially disperses the broadband optical pulse into a rainbow designed to capture 1D line scans of particles flowing through the channel. The temporal duration of these pulses is 27 ps (shutter speed), and they occur at a repetition rate of 36.7 MHz (line scan rate), while the average illumination power is 500 μW. The back-reflected pulses from the microfluidic device are directed via

![Fig. 1. STEAM flow analyzer. An animated video that shows the integrated functionality of the system is available (Movie S1). (A) Schematic of the STEAM flow analyzer that highlights the optical layout of the STEAM camera and real-time optoelectronic time-stretch image processor. The STEAM camera takes blur-free images of fast-flowing particles in the microfluidic device. The acquired images are optoelectronically processed and screened in the real-time optoelectronic time-stretch image processor. (B) Microfluidic device in which particles are controlled to flow at a uniform velocity and focused and ordered by inertial lift forces in the microfluidic channel. (C) Field-programmable digital image processor that captures particles and performs fully automated particle classification in real time. It consists of (i) a high-speed analog-to-digital converter (ADC); (ii) a field-programmable gate array (FPGA) for particle capture, E-slide generation, and coarse particle classification; (iii) an on-board memory circuit for storing selected E-slides; and (iv) a central processing unit (CPU) for fine particle classification.](https://www.pnas.org/content/109/24/10054.full)

Goda et al.
an optical circulator toward the real-time optoelectronic time-stretch image processor followed by the high-speed photodetector and the field-programmable digital image processor. Two-dimensional E-slides are then constructed from the digitized 1D frames and made available for screening.

As shown in Fig. 1A, the real-time optoelectronic time-stretch image processor consists of an amplified dispersive Fourier transform (29–32), a high-speed photodetector, and a field-programmable digital image processor. First, the amplified dispersive Fourier transformer serializes the image-encoded pulse (i.e., the 1D image frame) into a pixel stream that is slowed down in time so that it can be digitized and processed in real time. While it is being stretched in time, the image-carrying pulse is amplified in the optical domain through distributed Raman amplification in the time-stretch element. Raman amplification is chosen because it has a broad and flexible optical spectrum and also leads to low noise figure in the frequency-to-time mapping process (29–32). The optical image amplification (approximately 1,000x) before photon-to-electron conversion is critical as it enables high-throughput microscopy in the ultrashort exposure time (27 ps) without the need for a high-intensity illuminator. In addition, the ultrafast shutter speed freezes any motion of particles in high-speed flow, allowing for acquisition of blur-free images. The amplified 1D data stream is detected by the high-speed single-pixel photodetector, eliminating the need for a detector array and hence readout time limitations. After analog-to-digital conversion by a real-time digitizer, the data is processed in real time in the field-programmable digital image processor with a programmable multi-stage decision-making architecture (Fig. 1C and Movie S1 and SI Text) that employs a field-programmable gate array (FPGA) (33, 34), an on-board memory circuit, and a central processing unit (CPU). Specifically, the function of the FPGA is to identify the presence of particles (ignoring the unimportant space between the particles), generate E-slides for the particles, coarsely down-select the slides (i.e., the particles) through their morphological and biochemical markers, and store those selected in the memory. As a second-step screening process, the CPU performs fine particle classification on the stored slides with more stringent algorithms such as decision-tree classification and supervised learning methods (35, 36).

The STEAM flow analyzer’s blur-free image acquisition enables differentiation of particles from a heterogeneous population. Fig. 2 shows E-slides of fast-flowing particles of various species in the microfluidic device captured by the STEAM flow analyzer. Here the particles were controlled to flow at a uniform speed of 4 m/s, which corresponds to a throughput of 100,000 particles/s based on the volumetric flow rate. The high-speed motion of the particles was frozen by the ultrafast shutter speed (ultrashort exposure time) of the STEAM camera (27 ps) yet without sacrificing sensitivity due to the optical image amplification whereas the reduced sensitivity and motion blurs caused by the CMOS camera’s much lower shutter speed and lack of optical image amplification are evident. For further comparison, stationary particles of the same types on a glass slide were obtained under a conventional microscope with a CCD camera with a much longer exposure time (17 ms shutter speed, 1,280 × 1,024 pixels, no optical image gain). Despite the fact that the STEAM camera is many orders of magnitude faster than the CCD camera, the two cameras share similar image quality (i.e., sensitivity and resolution).

**Screening of Budding Yeast with the High-Throughput Imaging Flow Analyzer.** To show the utility of the STEAM flow analyzer, we used it to demonstrate high-throughput screening of *Saccharomyces cerevisiae*, commonly known as budding yeast. Budding yeast are important in food science, are used as a model for studying eukaryotic cell biology, and are engineered to produce protein therapeutics. Growth in yeast can be studied and optimized by flow cytometry or microscopy—both of which possess specific limitations. Biochemical analysis alone via flow cytometry is insufficient for analysis of complex checkpoints and detection of minor perturbations in the cell cycle (37) and provides poor characterization of the asymmetric growth of yeast in comparison with imaging (38). A high-throughput microscopy technique such as our STEAM flow analyzer could provide a powerful screening tool to assay morphological changes potentially accompanying systematic gene knockouts or other molecular perturbations.

The STEAM flow analyzer can efficiently identify and screen budding yeast cells and perform subpopulation analysis in real time. Here we programmed the FPGA and CPU to capture incoming cells, distinguish between budding and un budded cells, and classify budding cells based on different budding stage (Fig. 3A and SI Text). Specifically, the FPGA performs cell capture while ignoring the blank images and stores the corresponding E-slides in the on-board memory (1 GB) which can save up to approximately 16,000 v slides (each of which has 64/v KB), where v is the flow speed in units of m/s (Fig. S3A). Here because the 2D E-slides are constructed from a series of 1D frames with the second dimension provided by the flow, the size of each slide decreases, and hence the total number of slides that can be saved in the memory increases as the flow speed increases. We estimate

---

![Image](https://via.placeholder.com/150)

**Fig. 2.** Performance of the STEAM camera and comparison with a conventional CCD camera and a state-of-the-art CMOS camera. E-slides of flowing particles of various species in the microfluidic device were generated by the STEAM flow analyzer with the built-in STEAM camera (27 ps shutter speed, 128 × 512 pixels, 25 dB optical image gain). The E-slides are compared with images of the same particles captured by a state-of-the-art CMOS camera (1 μs shutter speed, 32 × 32 pixels, no optical image gain) under the same flow. To operate at these high speeds, the CMOS camera used partial read-out, limiting the number of pixels to 32 × 32. Here the particles were controlled to flow at a uniform speed of 4 m/s, which corresponds to a throughput of 100,000 particles/s based on the volumetric flow rate. The high-speed motion of the particles was frozen by the ultrafast shutter speed (ultrashort exposure time) of the STEAM camera (27 ps) yet without sacrificing sensitivity due to the optical image amplification whereas the reduced sensitivity and motion blurs caused by the CMOS camera’s much lower shutter speed and lack of optical image amplification are evident. For further comparison, stationary particles of the same types on a glass slide were obtained under a conventional microscope with a CCD camera with a much longer exposure time (17 ms shutter speed, 1,280 × 1,024 pixels, no optical image gain). Despite the fact that the STEAM camera is many orders of magnitude faster than the CCD camera, the two cameras share similar image quality (i.e., sensitivity and resolution).
that the flow speed can be set up to 8 m/s (corresponding to 200,000 cells/s) while retaining reasonable image quality and classification precision. The saved E-slides are then subject to the CPU’s classification process (Fig. S3B) in which the budding yeast cells are isolated and classified by daughter-to-mother ratio in size to generate a histogram for the subpopulations using a decision-tree classification method (Fig. 3F). For comparison, we also performed normal microscopic observation of yeast cells with a CCD camera and performed digital image processing similar to the automated classification on the CPU in the STEAM flow analyzer and found good agreement between our high-throughput method and the conventional method (Fig. S3C), suggesting that the STEAM flow analyzer is useful for analysis of yeast growth behavior with high statistical precision. Because the STEAM flow analyzer operates in real time, time-resolved statistical analysis of the subpopulations can also be performed to control and optimize the budding process. Furthermore, similar capture and subpopulation analysis can also be applied to emulsions for applications in cosmetics and pharmaceutics.

**Rare Cell Detection with the High-Throughput Imaging Flow Analyzer.**

To further show the utility of the STEAM flow analyzer, we used it to demonstrate rare cell detection. While statistically insignificant and hence often ignored, rare events among a large heterogeneous population of cells in blood such as hematopoietic stem cells (39), antigen-specific T cells (40), and circulating tumor cells (41, 42) (Table S4) are important in biomedical research as well as medical diagnostics and therapeutics (43). Such rare cells can be identified by a combination of morphological (i.e., size, circularity, and clustering) and biochemical (i.e., surface antigens) markers. Here our model for rare cells is the MCF7 cell line (breast cancer) spiked in blood. Red blood cells are lysed with a hypotonic lysing agent while MCF7 cells are fixed with formaldehyde and coated with metal beads with a diameter of 1 μm via an antibody to EpCAM (a cell surface molecule that exists on the surface of epithelial cells but not on the surface of blood cells). Our observation under a conventional microscope indicates that approximately 80% of MCF7 cells are coated with 5–20 metal beads (Fig. S2).

To demonstrate how surface marker detection could be achieved and coupled with morphological analysis in our system, we performed off-line statistical analysis of white blood cells and coated MCF7 cells. This validation is important because it is required to build a fully trained model for our supervised learning method or support vector machine (SVM) (35, 36) to be implemented on the FPGA and CPU (see SI Text). The SVM model predicts whether a new target (i.e., cell) falls into any of the predefined cell categories and is then used to identify the target cells in real time. Fig. 4A shows scatter plots of white blood cells and MCF7 cells based on the cell size (i.e., diameter) and presence of surface antigens (i.e., metal beads) produced by our system. The plots indicate that the system is capable of differentiating most white blood cells and MCF7 cells with high specificity using a single molecular marker. For comparison, Fig. S4A shows, using a conventional flow cytometer, scatter plots of the same cell types based on forward- and side-scattered light intensities as well as PerCP/Cy5.5-antiCD45 and FITC-antiEpCAM (fluorescence markers), which selectively bind to white blood cells and MCF7 cells, respectively (see SI Text). The scattered light intensity plots do not allow for differentiation of the two cell types due to the significant overlap between the two groups. Furthermore, the overlap in the fluorescence intensity limits the specificity of the instrument down to approximately 0.1% subpopulations— inadequate for identification of a cell type that is rarer than 0.1% of the population (Table S4).

With the fully trained cell classification model, the STEAM flow analyzer can detect MCF7 cells as rare as one in a million in a short period of time. Here we programmed the FPGA and CPU such that the FPGA captures incoming cells and performs size-based cell classification (Fig. S4B) while the CPU performs classification by circularity and presence of metal beads (see SI Text). The threshold for the size-based selection is set such that smaller MCF7 cells are also selected at the expense of detecting larger white blood cells (Fig. 4A). Yet, this process efficiently rejects more than 99.9995% of white blood cells, all residual red blood cells, and all free-floating metal beads, leaving only a small number of false positive events (on the order of 10 per mL of lysed blood) along with true positive events (Fig. 4B). The FPGA’s ability to filter out the large number of blood cells and avoid storing the massive amount of digital data enables nonstop real-time operation. The down-selected E-slides (i.e., cell images) are stored in the memory (1 GB) which can save up to approximately 4,000 v-slides (each of which has 256×K B), where v is the flow speed in units of m/s as described above. The stored slides are then subject to the CPU’s selection process in which the cells are further classified by circularity and presence of metal beads (Fig. S4C). This process rejects almost all the false positive events (i.e., multiple, unfocused, or unordered white blood cells) that have survived from the initial selection process on the FPGA while leaving true positive events and a very small number of false positive events (on the order of 10 per mL of lysed blood), which arise due to image processing artifacts and can further be rejected by human visual inspection (Fig. S4D).

Our statistical analysis of the capture efficiency indicates that the field-programmable digital image processor can identify
extremely rare cells with a high efficiency of 75% (limited by the imperfect coating efficiency and missing smaller MCF7 cells in the FPGA selection process) (Fig. 4C). Furthermore, our receiver operating characteristic (ROC) (44, 45) curve analysis of the results indicates that our method is sufficiently sensitive for detection of approximately one MCF7 cell in a million white blood cells and is 100 times better in terms of false positive rate than the conventional flow cytometer (Fig. 4D) yet without sacrificing throughput (see SI Text). Here all the measurements were performed at a throughput of 100,000 cells/s, corresponding to screening of 10 mL of lysed blood in less than 15 min.

Discussion

We have developed a high-throughput single-microparticle flow-through image analyzer for real-time image acquisition and screening of a large heterogeneous population of particles. By overcoming the technological limitations that exist in conventional automated microscopes (with many pixels and a throughput of approximately 1,000 particles/s) (Table S5) and high-throughput flow analyzers (with a single pixel and approximately 100,000 particles/s throughput), our system has achieved real-time image-based screening with high sensitivity (75%), high specificity (one in a million), and high statistical precision (approximately 100,000 particles/s simultaneously). Here the throughput is only limited by the microfluidic device’s tolerance to high pressures caused by high flow rates, not the STEAM camera’s image acquisition speed or the field-programmable digital image processor’s processing speed. We estimate that the throughput can be increased to 200,000 particles/s while retaining reasonable image quality and classification precision (Table S1). Our method can also be combined with a conventional cell sorter to sort out rare target cells for further genetic analysis of the cells. Furthermore, our method can, in principle, work at shorter wavelengths for higher spatial resolution, provided that proper optical components are available.

While in our proof-of-concept demonstration, we showed high-throughput screening of budding yeast and detection of rare breast cancer cells spiked in blood, our method should also be amenable to other applications in which high-throughput microscopy is required. Such applications include imaging and detection of bioparticles of interest in oceanography (e.g., phytoplanktons), energy science (e.g., oil emulsions and engineered microbes), environmental science, food science, cosmetics, pharmaceutics, and medicine (e.g., other rare cell types listed in Table S4).

Materials and Methods

The initial concentration of cells that were used for the rare cell detection measurements was determined by a hemacytometer. Four counts were performed and the mean and standard deviation were calculated. Clusters of
cells were counted as a single cell. The fate of these clusters in the microfluidic device is unclear; clusters could be filtered out upstream, traverse the channel as a cluster, or break up into smaller clusters or individual cells. The coated MCF7 cells were diluted into 3 mL of lysed blood. The resulting concentrations were 0, 5, 10, 50, 100, and 500 coated MCF7 cells per 3 mL. The standard deviation of the initial count is expected to decrease by the dilution factor for each spiked sample. Other sources of error, such as in the process of pipetting, are more difficult to quantify due to the lack of comparable technology for detecting extremely rare events or low concentrations of cells. The fit function used in Fig. 4C is \( y = ax + b \), where \( x \) and \( y \) are the spiked and captured numbers of MCF7 cells, respectively, \( a \) is the capture efficiency, and \( b \) is the contamination. For large \( x \), the fit function asymptotically approaches \( y \approx ax \). From the fit, the capture efficiency was found to be 75% with a coefficient of determination of 0.94, indicating that the regression line represents the data well.


ACKNOWLEDGMENTS. We thank T. Fukushima for valuable discussions, Y. Liu for assistance with the experiments, and L. Vandenberghhe for his suggestion to use the SVM. We acknowledge support from the U.S. Congressionally Directed Medical Research Programs (W81XWH1010519), the Microsystems Technology Office in the U.S. Defense Advanced Research Projects Agency, the National Institutes of Health (NIH), and Caltech-UCLA Joint Center for Translational Medicine. K. G. holds a Career Award at the Scientific Interface from the Burroughs Wellcome Fund. J. A. and C. W. are supported by the German Research Foundation and the Natural Sciences and Engineering Research Council of Canada, respectively. The flow cytometry experiment was performed in the Jonsson Comprehensive Cancer Center (JCCC) and Center for AIDS Research Flow Cytometry Core Facility at the University of California, Los Angeles (UCLA), that is supported by the NIH awards CA-16042 and AI-28697 and by the JCCC, the UCLA AIDS Institute, and the David Geffen School of Medicine at UCLA.

Supporting Information

Goda et al. 10.1073/pnas.1204718109

SI Text

Microfluidic Device Fabrication. The microfluidic device was fabricated in thermoset polyester (TPE) using standard replica molding methods (Fig. S1) (1). Our previously reported channels (2) were drawn in AutoCAD (Autodesk, Inc.). The design was printed as a photolithography transparency mask at 20,000 dots per inch (dpi). A negative photoresist (KMPR 1050, MicroChem, Corp.) was spun at 3,000 rotations per minute (rpm) on a 4-inch silicon wafer. The wafer was heated on a hot plate at 95 °C for 20 min, then exposed to UV light with a power of 8.0 mW/cm² through the transparency mask for 90 s. The wafer was baked again at 95 °C for 4 min then developed in SU-8 developer (MicroChem). The resulting master mold had a feature height of 43 μm. A replica was made by casting Silgard 184 Elastomer Kit (polydimethylsiloxane, PDMS, Dow Corning), mixed according to recipe, over the master.

Next, the replica was peeled off the master and PDMS mold of this replica was made as described by Kuo et al. (3). Briefly, the PDMS replica was oxidized in air plasma then silanized with 0.2% octadecyltrichlosilane (Sigma-Aldrich) in ethanol for 15 min. It was then rinsed with ethanol and heated to 65 °C for 30 min to prevent swelling. PDMS was then cast on the PDMS replica to create a mold with the same polarity as the master. It was degassed and cured at 65 °C for 2 hr. This mold was separated from the replica and sonicated in isopropanol for 5 min, sonicated in deionized H₂O for 5 min, and baked at 65 °C for at least 30 min. Holes were punched for tubing connectors.

TPE channels were fabricated following the protocol of Fiorini et al. (4). First, 0.10 g of the UV-phothoinitiator (2,2-dimethoxy-2-phenylacetophenone, Irgacure 651, Crompton Corp.) and 0.99 g of methyl-ethyl-ketone-peroxide catalyst (MEKP, Crompton Corp.) were added. The mixture was stirred then degassed for 5 min. PEEK (Polyetheretherketone) tubing segments were inserted into the PDMS mold to provide a molded region for tubing connectors to be inserted later. The TPE mixture was cast over the PDMS mold to a thickness of 3 mm and covered with transparency film (3 M), cut to an appropriate size to ensure a flat, top surface (Fig. S2). The TPE mixture was cast over the PDMS mold to provide a molded region for tubing connectors (Polyetheretherketone) tubing segments were inserted into the mold to a thickness of 3 mm and covered with transparency film (3 M), cut to an appropriate size to ensure a flat, top surface (Fig. S2). The TPE mixture was cast over the PDMS mold to provide a molded region for tubing connectors (Polyetheretherketone) tubing segments were inserted into the mold to a thickness of 3 mm and covered with transparency film (3 M), cut to an appropriate size to ensure a flat, top surface (Fig. S2).

Illumination and Imaging Optics of the STEAM Camera. The optical source is a mode-locked femtosecond pulse fiber laser with a pulse repetition rate of 36.7 MHz. After supercontinuum generation in a highly nonlinear fiber and band-pass filtering, a nearly flat spectral shape with 20 nm bandwidth centered at 1,590 nm is produced for target illumination. A pair of diffraction gratings with 1,100 lines/mm spatially disperses the pulses along a 1D line, producing 1D rainbow pulses.

Amplified Dispersive Fourier Transformer. The amplified dispersive Fourier transformer (ADFT) consists of a dispersive fiber with −1,200-ps/nm dispersion pumped by four continuous-wave lasers with approximately 100 mW at 1,470 nm, 1,480 nm, 1,480 nm, and 1,490 nm for distributed Raman amplification (5–8). Before the ADFT, an erbium-doped fiber amplifier (EDFA) is used as a pre-amplifier. The EDFA and ADFT produce a net gain of up to 13 dB and 17 dB (depending on signal-to-noise ratio, respectively). The specifications of the STEAM camera can be estimated from the parameters of its components. First, the number of 1D image pixels on the target (N) is found from the total dispersion in the dispersive fiber (D = −1,200 ps/nm), the optical bandwidth (Δλ = 20 nm), and the sampling rate of the ADC in the field-programmable digital image processor (f_s = 7 GS/s) to be N = D · Δν · f_s = 168 (which is reduced down to 128 by removing the frame edges) whereas the number of 1D resolvable points is about 200 from the spectral resolution of the ADFT process. Second, the spatial resolution of the STEAM camera is found to be about 1.4 μm from the illumination and imaging optics. Finally, the shutter speed (exposure time) is estimated from the bandwidth of each rainbow pulse (20 nm/200) and the time-bandwidth product to be 27 ps (assuming that each sub-pulse of the rainbow pulse is near transform limited).

Sensitivity Analysis. From the power of the illumination (500 μW) incident onto an area whose size is 30 μm × 2 μm, the illumination intensity is 8.3 W/mm² (well below the damage threshold of biological samples). Due to the optical loss in the imaging optics, fiber coupler, and diffraction gratings, about 5 μW is returned to the optical image amplifier that consists of an EDFA (up to 13 dB of gain) and distributed Raman amplifier (up to 17 dB of gain) before the photodetector with a conversion gain of −850 V/W and a noise-equivalent power (thermal noise) of 24 pW/Hz¹/₂. Then, the voltage noise power of the signal is given by ([−850 V/W] × (5 μW)]²/(50 Ω) = 361 nW, where 50 Ω is the transimpedance of the photodetector, which is two orders of magnitude below the thermal noise power of 42 μW using a detection bandwidth of 3 GHz. Therefore, without optical image amplification, the signal (image) could not be detected. The optical amplifier amplifies the signal by 30 dB from 361 nW to 361 μW, and then the signal-to-noise ratio becomes 1.4 μm from the illumination and imaging optics. Finally, the shutter speed (exposure time) is estimated from the bandwidth of each rainbow pulse (20 nm/200) and the time-bandwidth product to be 27 ps (assuming that each sub-pulse of the rainbow pulse is near transform limited).

CMOS and CCD Cameras. The CMOS camera used in Fig. 2 is Phantom v7.3 (Vision Research). It has an exposure time (shutter speed) of 1 μs and a pixel number of 32 × 32. The CCD camera used for Fig. 2 and Fig. S2 is DS-Qi1 with a DS-U2 PC-based control unit (Nikon). It has an exposure time (shutter speed) of 17 ms and a pixel number of 1,280 × 1,024.
Architecture of the Field-Programmable Digital Image Processor. The field-programmable digital image processor employs a programmable multi-stage decision-making model that can be tailored, depending on the application. While in this paper we demonstrated screening of budding yeast, oil emulsions in water, and rare breast cancer cells in blood, the capability of the image processor is not limited to these applications, provided that proper algorithms are available. The field-programmable digital image processor consists of the ADC, FPGA, memory circuit, and CPU. The structure and function of the FPGA (coarse) and CPU (fine) classification processes are schematically shown in Figs. S3 and S4.

The FPGA classification process is described as follows. First, the digitized output of the ADC sampled at 7 GS/s by time interleaving is inputted to the on-board Xilinx Virtex-6 FPGA device (LX240T). The FPGA runs at a frequency of 200 MHz and receives 32 samples of time-interleaved data at every clock cycle. The incoming data is synchronized in the FPGA by selecting four clock cycles of data from the first positive sample point value in the rising edge of the pulse (i.e., the 1D image frame), which forms one frame of the image (i.e., 128 sample points per frame). Initially, the fourth incoming frame is selected as the reference frame by the FPGA and it is done while PBS is passed through the microfluidic channel. Once the reference frame has been selected by the FPGA, the incoming frames are checked for meeting criteria of the target particle. The criteria check consists of comparing the difference between the value of sample points in the incoming frame and the corresponding points in the reference frame to a threshold value which matches to the value observed at the center of the typical target particle, which is different from the other untargeted particles. To represent an entrance frame to a threshold value which matches to the value in the incoming frame and the corresponding points in the reference frame to a threshold value which matches to the value observed at the center of the typical target particle, which is different from the other untargeted particles. To represent an entrance frame to a threshold value which matches to the value observed at the center of the typical target particle, which is different from the other untargeted particles. To represent an entrance frame to a threshold value which matches to the value observed at the center of the typical target particle, which is different from the other untargeted particles.

While criteria checking on an incoming quarter frame (32 samples) is taking place at a clock cycle, the quarter frames are stored into the memory bank 0 which is a 256 × 4,096/v block RAM in the FPGA. The FPGA can store 1,024/v frames as 4,096/v quarter block RAM in the FPGA while the pixel image frames stored in the bank 0 is transferred to the on-board 1 GB DDR RAM. After completion of the frame transfer from bank 0, the input to the external DDR RAM is multiplexed to the output of memory bank 1 to transfer the frames stored in it and simultaneously the incoming frames are checked for the target particle and gets written to memory bank 0 as explained before.

After the initial filtration process on the FPGA captures nearly all of the target particles along with occasional suspicious-looking untargeted particles, their images are transferred to the CPU for further processing. A particle classification algorithm is prepared by studying the images of a large number of particles from each particle type to identify key differences in their signatures. This further particle filtration is implemented off-line on the captured particles to differentiate similar-looking target particles and untargeted particles based on more delicate differences of their images. This efficient and accurate image processing algorithm would require more processing time and needs to be implemented as a post-processing step on a more powerful processor than the FPGA. It takes advantage of a large library of particles each marked as belonging to one of two or more categories. The supervised learning algorithm analyzes the training data and produces a model that maps each image into a 2D space such that particles from different categories are separated in this space. New examples from mixed particle samples that are captured and prefiltered by the FPGA are then mapped into that same space and predicted to belong to a category based on their location in this space.

In the budding yeast screening experiment, the flowchart shown in Fig. S3 is used for cell classification. Initially, the cell images are specifically thresholded. The resulting images only contain binary information representing the cell geometry. As a second step, this 2D geometry is mapped to a 1D diameter profile where the maxima directly represent twice the radius of the detected cells. In order to properly detect the maxima, the 1D data is smoothed by correlating it with a Gaussian profile that exhibits a half width of the approximate radius of the mother cell. This followed by a tree-based decision process that determines whether a singlet (one maximum) or budded cell (two maxima) is present. In the latter case, the amount of the detected maxima is used to create a volume ratio. The detected volume ratios are finally gathered to create the daughter-to-mother ratio histogram.

In the rare cell detection experiment, the SVM concept shown in Fig. S4 is used for cell classification. During the training process, the SVM algorithm defines and optimizes key features based on size, metal content, and circularity of cells. Metal content is correlated to the introduction of regions with strong shadows on the cell image due to attachment of the metal beads. It is a good measure of surface antigens, which are specific to target MCF7 cells. On the other hand, the exact size of the cell from its image serves as an extra measure of differentiating the two cell types, as MCF7 cells are on average larger than white blood cells. Furthermore, circularity of cells is also evaluated as another measure of cell differentiation since white blood cells are typically circular or symmetrically shaped while MCF7 cells are irregularly or asymmetrically shaped or clustered with a few to several MCF7 cells. The optimized SVM utilizing size, metal content, and circularity can identify rare MCF7 cells with a false positive rate of approximately one in a million.

In the size-based selection process, 10–20% of MCF7 cells are missed, depending on the total number of MCF7 cells in the sample (if the number is small, the percentage fluctuates due to statistical fluctuations). Even if very small MCF7 cells survive from the size-based selection process, they tend not to express metal beads via their surface antigen because their surface area is smaller (the number of metal beads on the MCF7 cell is proportional to the surface area). Therefore, the coating efficiency is somewhat coupled to the size of MCF7 cells. Consequently, our statistical analysis of the aggregate capture efficiency indicates that the size-based screening along with the metal- and circularity-based check implemented on the FPGA and CPU can identify rare MCF7 cells with a high efficiency of 75%, meaning that only 25% of the cells are missed.

Preparation of Oil Emulsions in Water. A mixture of 500 cSt PDMS 200 fluid (3% v/v) and Tween 80 (3% v/v) was prepared in di-ionized water. The mixture was vortexed heavily to create polydisperse emulsions.

Budding Yeast Screening Experiment. Yeast was cultured in tryptic soy broth (TSB) in an incubated shaker (37 °C) for two days prior to imaging. For imaging, the cultured suspension was diluted in phosphate buffered saline (PBS).

Rotational Behavior of Budding Yeast. The rotational behavior of the budding yeast in this flow is a concern due to how it may impact image quality. Videos recorded with the Phantom v7.3 high-speed camera reveal the rotation of yeast in this flow appears similar to classic Jeffery orbits (9), in agreement with observations of polymeric sphere doublets in similar flow conditions (10). In that study, it was found that elongated particles re-
sided longer with their longer axis of symmetry parallel to the flow direction than with their longer axis of symmetry perpendicular to the flow direction, and the residence time in the parallel orientation increased with increasing length of the doublets. Therefore, we should expect some yeast buds to be eclipsed sometimes, but they should predominantly be exposed while passing through the line scan. If error due to this phenomenon is a concern, the microfluidic channel could be designed such that the shear gradient is oriented in the perpendicular direction. This would result in all buds un eclipsed except when one bud is too small to impact the rotational axis.

Protocol for Coating MCF7 Cells with Metal Beads. MCF7 cells were harvested from culture and incubated in 4% formaldehyde for 10 min. They were then resuspended and incubated in 1 mL PBS with Anti-Human CD326 (EpCAM) Biotin (13-9262-80; Ebioscience, Inc.). The antibody-coated cells were washed twice with PBS then suspended in 1 μm, hydrophilic, streptavidin-coupled, superparamagnetic beads (650-01; Invitrogen Corporation, Carlsbad, CA) and mixed thoroughly.

Flow Cytometry Experiment. MCF7 cells were harvested and fixed in 0.5% formaldehyde for 10 min, washed and resuspended in PBS. Whole blood was mixed with red blood cell lysis buffer (Roche) at a ratio of 2 (lysis buffer) to 1 (whole blood) and incubated for 10 min. The remaining white blood cells were resuspended once in lysis buffer, then in PBS. Both MCF7 cell and white blood cell suspensions were incubated with FITC-antiEpCAM and PerCP/Cy5.5-antiCD45. Unbound dyes were removed after 30 min and cells were washed and resuspended in PBS. The pure MCF7 and white blood cell suspensions and two mixtures were analyzed with the FACSCalibur system in the Flow Cytometry Core Laboratory at UCLA.

Receiver Operating Characteristic (ROC) Curve Analysis. To generate an ROC curve (11, 12), we convert a 2D scattering space into a table of true positive rate (TPR) with respect to false positive rate (FPR). Then, at each FPR point, we use a discrimination threshold for a 2D scattering space, and hence the only way to be maximized is to produce the maximum TPR. This is important in order to compensate for the reduced sensitivity at high frame rates. Furthermore, the ImageStream is not capable of detecting rare cell types which are rarer than approximately 0.01% of the total population with high statistical precision (equivalently at the level of circulating endothelial cells and rarer cell types, described in Table S4). Because cell detection obeys Poisson statistics, its statistical precision improves with \( \sqrt{N} \), where \( N \) is the number of rare cells detected by the instrument. Detecting cells with are as rare as 1 in 10 million (corresponding to the number of circulating tumor cells in lysed blood) with reasonable statistical precision would take weeks with the ImageStream due to its limited throughput and cell count and the lack of on-line real-time image processing. With a throughput of 200,000 cells/s and the power of our real-time optoelectronic image processor, our system can process the same sample in less than 10 min.


Fig. S1. Procedure for the microfluidic device fabrication. From steps 1 to 4, a KMPR-on-silicon master mold is fabricated by photolithography. From steps 5 to 7, a PDMS replica is cured on the master mold and then allows the fabrication of a second PDMS mold with the same polarity as the KMPR-on-silicon master. From steps 8 to 10, tubings are inserted to define inlets and TPE is semi-cured over the PDMS mold. From steps 11 to 14, the TPE replica is removed from the mold, bonded to the substrate, and cured. Finally, from steps 15 to 16, injection tubings are connected to the chip and glued for high injection pressures.
Fig. S2. CCD images of stationary particles. Images of particles of various species were taken by a research-grade CCD camera. The exposure time (shutter speed) and pixel number of the CCD camera are 17 ms and 1,280 × 1,024, respectively.

Fig. S3. Supporting information for screening of budding yeast. (A) Block diagram of the hardware implemented on the FPGA for the budding yeast screening. The FPGA performs cell capture and stores E-slides for the cells on the memory. The file size shown in the diagram (and hence the number of E-slides that can be saved on the memory) varies, depending on the flow speed. The values specified in the diagram correspond to the case of v = 1 m/s. (B) Decision-tree classification algorithms implemented on the CPU for the budding yeast screening. The CPU performs a tree-based cell radius dependent decision scheme to distinguish between budding and unbudded cells and create a histogram based on daughter-to-mother ratio in volume. (C) Subpopulation analysis of budding yeast with a conventional method. The histogram was generated by normal microscopic observation of yeast cells with a CCD camera and digital image processing similar to the automated classification on the CPU in the STEAM flow analyzer. The results indicate that the STEAM flow analyzer is useful for analysis of yeast growth behavior with high statistical precision.
Fig. S4. Supporting information for rare cell detection. (A) Conventional flow cytometer’s scatter plots. Shown are events of white blood cells and MCF7 cells coated with 1-μm metal beads based on forward- and side-scattered light intensities as well as events of white blood cells and MCF7 cells coated with PerCP/Cy5.5-antiCD45 and FITC-antiEpCAM (fluorescence markers), which selectively bind to white blood cells and MCF7 cells, respectively. The total number of events is approximately 10,000 for both cell types. The scattered light intensity plots are inadequate for differentiation of the two cell types due to the significant overlap between the two distributions. Furthermore, overlaps in the fluorescence scatters due to unwanted auto-fluorescence, nonlinear optical processes, and detector noise limit the specificity of the instrument down to approximately 0.1% of the total population—inadequate for identification of a cell type that is rarer than approximately 0.1% of the population (Table S4). (B) Block diagram of the hardware implemented on the FPGA for the rare cell detection. The FPGA performs cell capture and size-based cell classification. The file size shown in the diagram (and hence the number of E-slides that can be saved on the memory) varies, depending on the flow speed. The values specified in the diagram correspond to the case of v = 1 m/s. This classification process filters out nearly 99.9995% of white blood cells, all residual red blood cells, and all free-floating metal beads, leaving only false positive events of the order of 100 per mL of lysed blood along with true positive events. (C) SVM implemented on the CPU for the rare cell detection. This cell filtration process is performed off-line on the captured cells by the FPGA to differentiate similar looking MCF7 cells and white blood cells based on more delicate differences of their images. The cell classification program uses a SVM to classify the images. The optimized SVM utilizing size, metal content, and circularity (including clustering) can identify rare MCF7 cells with a false positive rate of approximately one in a million. (D) E-slides of free-floating metal beads, white blood cells, and metal-coated MCF7 cells captured by the STEAM flow analyzer. These E-slides are used to train the SVM.
**Movie S1.** Animated video that shows the functionality of the STEAM flow analyzer. Broadband optical pulses generated by the optical source are spatially dispersed by a pair of diffraction gratings into 1D rainbow pulses which are incident onto the microfluidic channel. Dominant background particles and rare target particles coated with metal beads are controlled to flow at a uniform velocity and focus and order due to inertial forces in the channel. The rainbow pulses are incident onto the particles via the objective lens, illuminating different spatial coordinates along the horizontal axis with different frequency components of the rainbow pulses. The image of the incoming particle is imprinted onto the spectrum of the pulses. The back-reflected rainbow pulses return to the same optics and directed via the optical circulator toward the amplified dispersive Fourier transformer (i.e., optical image amplifier and serializer) where the pulses are serialized and amplified in the optical domain. The amplified data steam is detected by the single-pixel photodetector and transferred to the FPGA, which detects and captures the presence of the particles in the field of view (while ignoring the gaps between particles), generates E-slides for the particles, and performs coarse particle classification. The down-selected E-slides are sent to the computer (i.e., the CPU) in which fine particle classification is performed, and then displayed on the monitor. The investigator further classifies the E-slides by visual inspection.

**Movie S1 (MOV)**

---

**Table S1. Specifications of the STEAM flow analyzer**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput (corresponding flow speed)</td>
<td>50,000 particles/s (2 m/s)</td>
</tr>
<tr>
<td></td>
<td>100,000 particles/s (4 m/s)</td>
</tr>
<tr>
<td></td>
<td>200,000 particles/s (8 m/s)</td>
</tr>
<tr>
<td>Frame rate</td>
<td>36.7 MHz (line scan mode)</td>
</tr>
<tr>
<td>Shutter speed</td>
<td>27 ps</td>
</tr>
<tr>
<td>Flow technology</td>
<td>inertial focusing and ordering</td>
</tr>
<tr>
<td>Flow speed</td>
<td>up to 8 m/s</td>
</tr>
<tr>
<td>Optical image gain</td>
<td>up to 30 dB</td>
</tr>
<tr>
<td>Sampling rate</td>
<td>7 GS/s</td>
</tr>
<tr>
<td>Pixel number (corresponding flow speed)</td>
<td>For smaller particles</td>
</tr>
<tr>
<td></td>
<td>• 128 x 256 (2 m/s)</td>
</tr>
<tr>
<td></td>
<td>• 128 x 128 (4 m/s)</td>
</tr>
<tr>
<td></td>
<td>• 128 x 64 (8 m/s)</td>
</tr>
<tr>
<td></td>
<td>For larger particles</td>
</tr>
<tr>
<td></td>
<td>• 128 x 1,024 (2 m/s)</td>
</tr>
<tr>
<td></td>
<td>• 128 x 512 (4 m/s)</td>
</tr>
<tr>
<td></td>
<td>• 128 x 256 (8 m/s)</td>
</tr>
<tr>
<td>Field of view</td>
<td>30 x 30 μm²</td>
</tr>
<tr>
<td>Laser center wavelength</td>
<td>1,590 nm</td>
</tr>
<tr>
<td>Optical bandwidth</td>
<td>20 nm</td>
</tr>
<tr>
<td>Illumination power</td>
<td>500 μW</td>
</tr>
<tr>
<td>Numerical aperture</td>
<td>0.65</td>
</tr>
<tr>
<td>Image analysis</td>
<td>online real time</td>
</tr>
</tbody>
</table>

The throughput is limited by the microfluidic device’s tolerance to high pressures caused by high flow rates, not the STEAM camera’s shutter speed and frame as well as the real-time optoelectronic time-stretch image processor’s processing speed. For reasonable image quality and screening precision, the system can operate at a throughput of 200,000 particles/s.
Table S2. Specifications of the microfluidic device

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions of microfluidic device</td>
<td>112 μm (width)</td>
</tr>
<tr>
<td></td>
<td>43 μm (height)</td>
</tr>
<tr>
<td></td>
<td>2.2 cm (length)</td>
</tr>
<tr>
<td>Volumetric flow rate (corresponding mean channel velocity)</td>
<td>0.6 mL/min (2 m/s)</td>
</tr>
<tr>
<td></td>
<td>1.2 mL/min (4 m/s)</td>
</tr>
<tr>
<td></td>
<td>4.8 mL/min (8 m/s)</td>
</tr>
<tr>
<td>Channel material</td>
<td>thermoset polyester</td>
</tr>
<tr>
<td>Maximum pressure</td>
<td>≥150 psi</td>
</tr>
<tr>
<td>Young's modulus</td>
<td>1.2 GPa</td>
</tr>
<tr>
<td>Light transmittance</td>
<td>90%</td>
</tr>
<tr>
<td>Swelling ratio in ethanol</td>
<td>1.0 (no swelling)</td>
</tr>
</tbody>
</table>

The device was fabricated in thermoset polyester to ensure channel rigidity and hence stability in the real-time cell classification. Channel rigidity is particularly important because if the channel deforms, it can cause undesirable fluid dynamic and optical effects. Other advantages of thermoset polyester include its high stiffness, ease of fabrication, ability to sustain high pressures, and resistance to ethanol which helps channel washing.

Table S3. Specifications of the field-programmable digital image processor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPGA device</td>
<td>Xilinx Virtex-6 LX240T</td>
</tr>
<tr>
<td>FPGA clock frequency</td>
<td>200 MHz</td>
</tr>
<tr>
<td>Frame size</td>
<td>128 bytes (pixels/samples)</td>
</tr>
<tr>
<td>Samples received in one clock cycle</td>
<td>32 bytes (quarter frame)</td>
</tr>
<tr>
<td>E-slide size captured in FPGA</td>
<td>For smaller particles</td>
</tr>
<tr>
<td></td>
<td>• 512/v frames consisting of 256/v frames in two memory banks of block RAM (v = flow speed in units of m/s)</td>
</tr>
<tr>
<td></td>
<td>For larger particles</td>
</tr>
<tr>
<td></td>
<td>• 2,048/v frames consisting of 1,024/v frames in two memory banks of block RAM (v = flow speed in units of m/s)</td>
</tr>
<tr>
<td>External memory (DDR RAM) for storing E-slides</td>
<td>1 GB</td>
</tr>
<tr>
<td>Memory size of single E-slide</td>
<td>• 64/v KB for smaller particles</td>
</tr>
<tr>
<td></td>
<td>• 256/v KB for larger particles</td>
</tr>
<tr>
<td>Maximum number of E-slides stored in DDR RAM</td>
<td>• 16,000 v for smaller particles</td>
</tr>
<tr>
<td></td>
<td>• 4,000 v for larger particles</td>
</tr>
</tbody>
</table>

The FPGA device used in our system is a state-of-the-art FPGA (Xilinx Virtex-6 LX240T). The E-slide size captured in the FPGA can be tailored, depending on the size of the particles of interest.

Table S4. Blood components and their concentrations

<table>
<thead>
<tr>
<th>Blood component</th>
<th>Concentration (Ccells/mL of blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>5,400,000,000</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>350,000,000</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>6,000,000</td>
</tr>
<tr>
<td>T Lymphocytes</td>
<td>1,500,000</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>1,000,000</td>
</tr>
<tr>
<td>B Lymphocytes</td>
<td>600,000</td>
</tr>
<tr>
<td>Monocytes</td>
<td>500,000</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>250,000</td>
</tr>
<tr>
<td>Natural killer cells</td>
<td>200,000</td>
</tr>
<tr>
<td>Basophils</td>
<td>50,000</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>20,000</td>
</tr>
<tr>
<td>Hematopoietic stem cells</td>
<td>2,000</td>
</tr>
<tr>
<td>Antigen-specific T cells</td>
<td>1,000</td>
</tr>
<tr>
<td>Circulating endothelial cells</td>
<td>500</td>
</tr>
<tr>
<td>Fetal cells in maternal blood</td>
<td>500</td>
</tr>
<tr>
<td>Endothelial progenitor cells</td>
<td>200</td>
</tr>
<tr>
<td>Circulating tumor cells</td>
<td>10</td>
</tr>
</tbody>
</table>

1 mL of blood contains about 5.4 billion red blood cells (erythrocytes), about 350 million platelets (thrombocytes), and about 8 million white blood cells (neutrophils, lymphocytes, monocytes, etc). While rare events are commonly defined as subpopulations that occur at less than 5%, several types of cells are vanishingly rare. For example, the population of circulating tumor cells can number only a few parts per billion of normal blood cells.

Table S5. Comparison between the ImageStream and STEAM flow analyzer

<table>
<thead>
<tr>
<th></th>
<th>ImageStream (Amnis Corporation)</th>
<th>STEAM flow analyzer (University of California, Los Angeles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput</td>
<td>Up to 2,000 cells/s</td>
<td>Up to 200,000 cells/s</td>
</tr>
<tr>
<td>Image processing</td>
<td>off-line</td>
<td>on-line real-time</td>
</tr>
<tr>
<td>Cell count</td>
<td>50 μL of blood due to the limited storage and data access rate</td>
<td>Virtually limitless due to real-time optoelectronic image processing (3 mL of blood was used in our manuscript while it can be more than 1 L)</td>
</tr>
<tr>
<td>Cell capture efficiency</td>
<td>50% (Half the flowing cells are missed)</td>
<td>100% (All the flowing cells are captured)</td>
</tr>
<tr>
<td>Optical image gain</td>
<td>No gain (Poor SNR at high throughput)</td>
<td>1,000x (30 dB) (High SNR without sacrificing throughput)</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Morphology, fluorescence labeling</td>
<td>Morphology, microparticle labeling</td>
</tr>
<tr>
<td>Spatial resolution</td>
<td>1.0 μm at 2,000 cells/s</td>
<td>1.4 μm at any throughput up to 200,000 cells/s</td>
</tr>
<tr>
<td></td>
<td>0.5 μm at 1,000 cells/s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 μm at 600 cells/s</td>
<td></td>
</tr>
</tbody>
</table>

The commercially available imaging flow cytometer ImageStream by Amnis Corporation (1) [whose development was supported by others’ earlier work (2, 3)] is not capable of detecting rare cell types which are rarer than approximately 0.01% of the total population with high statistical precision (equivalently at the level of circulating endothelial cells and rarer cell types, described in Table S4). Detecting cells with are as rare as 1 in 10 million (corresponding to the number of circulating tumor cells in lysed blood) with reasonable statistical precision would take weeks with the ImageStream due to its limited throughput and cell count and the lack of on-line real-time image processing. With a throughput of 200,000 cells/s and the power of our real-time optoelectronic image processor, our system can process the same sample in less than 10 min.