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ULTRAFAST AUTOMATED IMAGE CYTOMETRY FOR CANCER DETECTION

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ABSTRACT

We present a method for ultrafast automated single-cell optical microscopy that performs blur-free image acquisition and non-stop real-time image-recording and classification of cells during high-speed flow. This method is based on a unique integration of ultrafast optical imaging, self-focusing microfluidics, optoelectronics, and information technology. To show the system's utility, we demonstrate high-throughput image-based screening of rare breast cancer cells in blood with an unprecedented throughput of 100,000 cells/s and a record false positive rate of one in a million. This method is expected to be effective for early, noninvasive, low-cost detection of cancer.

1. INTRODUCTION

In super-aged countries such as Japan, one of the biggest problems that strain and consume their resources is high medical costs [1]. In particular, diagnosis and therapy of cancer (the elderly's largest cause of death in Japan) take a tremendous portion of the medical costs since they require frequent diagnosis and long-term evaluation of cancer patients during chemotherapy with medical imaging (i.e., MRI, CT, and PET). While conventional medical imaging has been successful for identification of cancer tumors in a minimally invasive manner, a new type of technology is clearly needed to reduce the medical costs and hence help improve cancer patients' (in particular, the elderly's) quality of life and restore the national resources.

The main reason for the high medical costs in cancer diagnosis and therapy is, at the most fundamental level, the metastatic nature of cancer. In fact, statistics shows that 90% of cancer deaths is caused by cancer metastasis, not the original tumor [2-4]. Metastasis occurs in the body via so-called circulating tumor cells (CTCs) – the type of cells that detach from a primary tumor, circulate in the bloodstream, move to a new location, and create a secondary tumor. Therefore, CTCs may constitute seeds for the subsequent growth of additional tumors in different tissues, leading to long-term burden on the elderly both physiologically and financially. However, each CTC has a low chance of seeding secondary tumors, and hence CTCs are present well before a new tumor has appeared or been detected by conventional medical imaging techniques. Therefore, identification of CTCs provides an opportunity to detect precursors to metastasis and to prevent the spread of cancer through chemo or target-specific drug therapies.

Blood Component	Concentration (cells / mL of blood)
Erythrocytes	5,400,000,000
Thrombocytes	350,000,000
Neutrophils	6,000,000
T Lymphocytes	1,500,000
CD4+ T cells	1,000,000
B Lymphocytes	600,000
Monocytes	500,000
Eosinophils	250,000
Natural Killer Cells	200,000
Basophils	50,000
Dendritic Cells	20,000
Hematopoietic Stem Cells	2,000
Antigen-Specific T Cells	1,000
Circulating Endothelial Cells	500
Fetal Cells in Maternal Blood	500
Endothelial Progenitor Cells	200
Circulating Tumor Cells (CTCs)	**10**

Table 1: Blood components and their concentrations. The concentration of CTCs is only about 10 cells per mL of blood, meaning that they number only a few in a billion hematological cells and are hence extremely difficult to detect.

While detection of CTCs is obviously important for diagnosis and monitoring of cancer, they are exceedingly rare and hard to capture (Table 1). Their population can number only a few in a billion normal blood cells. In fact,

finding them is a problem that evokes the proverbial adage "finding a needle in the haystack." Without a doubt, detection of CTCs with good statistical accuracy in a practical period of time requires a high-throughput technique that can sift through an enormous population of blood cells.

While conventional flow cytometers (e.g., Coulter counters and fluorescence-activated cell sorters) provide high-throughput classification capability, they are incapable of detecting CTCs due to their high false positive rate (typically limited to ~0.1%), meaning that they cannot detect any rare cell types which are rarer than ~0.1% of the total population. This is caused by their inability to provide spatial metrics, rendering the instruments unable to resolve single, multiple, and clustered cells or unusually shaped cells as well as to distinguish debris and nonspecific labeling, leading to a large number of false positive events and inaccurate subpopulation counts.

2. ULTRAFAST AUTOMATED OPTICAL MICROSCOPY

An emerging technology that addresses the need for high-throughput detection of CTCs with high statistical accuracy is ultrafast automated optical microscopy that performs sensitive blur-free image acquisition and non-stop real-time image recording and classification of cells during high-speed flow [5]. This is made possible by integrating ultrafast optical imaging technology known as serial time-encoded amplified microscopy (STEAM) [6], inertial microfluidics, optoelectronics, and information technology. This high-throughput automated microscopy system holds great promise for early, noninvasive, and low-cost detection of cancer and evaluation of chemotherapy and will hence usher in a new era in pathology.

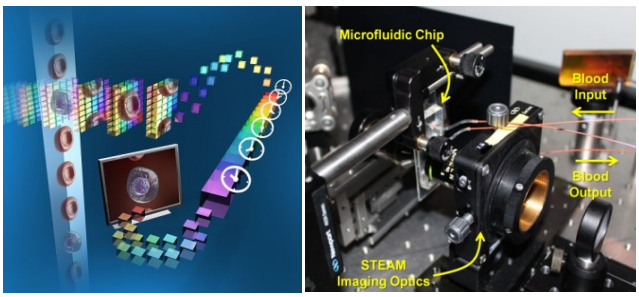

Figure 1: STEAM automated image cytometry. Left: conceptual schematic. Right: STEAM imaging optics and microfluidic chip. The STEAM automated image cytometer performs sensitive blur-free image acquisition and non-stop real-time image recording and classification of cells during high-speed flow.

This technology known as the STEAM automated image cytometer (Figure 1) consists of three key components: (1) an ultrafast optical imager based on STEAM, (2) a self-focusing microfluidic chip, and (3) a real-time digital image processor. The STEAM camera performs blur-free imaging of cells in high-speed flow. The self-focusing microfluidic chip employs inertial microfluidic technology for sheath-free focusing and ordering of cells with inertial forces. The real-time digital image processor performs real-time identification and classification of cells in the digital domain. The integrated system transforms cells in well-controlled microfluidic flow into a series of images on which cells of interest are digitally analyzed. With the power of high-speed digital signal processing techniques, this property enables fully automated real-time image-recording and classification of a large heterogeneous population of cells through their morphological and biochemical features. Consequently, the technology can evaluate, analyze, and screen rare CTCs with high specificity, high sensitivity, and high statistical accuracy in a short period of time.

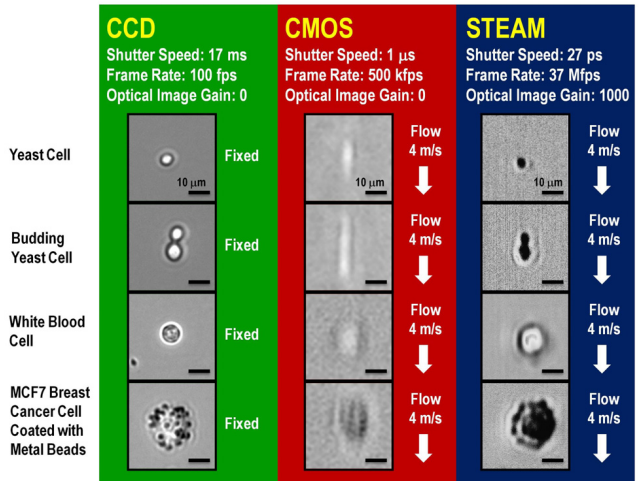

Figure 2: Comparison between the STEAM camera and conventional CCD and CMOS cameras. The ultrahigh shutter speed and optical image gain of the STEAM camera enable blur-free imaging of cells in high-speed flow. The STEAM camera operates nine orders of magnitude faster than the CCD camera yet with reasonable sensitivity.

Originally designed and demonstrated by Keisuke Goda, Kevin K. Tsia, and Bahram Jalali at University of California, Los Angeles [6], STEAM is a new type of imaging technology that overcomes both fundamental and technological limitations in conventional cameras such as CCD and CMOS image sensors. STEAM employs optical image amplification to circumvent the fundamental trade-off between sensitivity and speed – a predicament in virtually all optical imaging systems [6-8]. Critical for biological applications, STEAM's ability to amplify images in the "optical" domain enables high-speed microscopy at low light levels. It also performs photonic time stretch to slow down the image stream, allowing it to be captured by a single-pixel photodiode and digitized and processed in real time. This property eliminates the need for the detector array and readout time limitations.

Avoiding this problem and featuring the optical image amplification for dramatic improvement in sensitivity at high image acquisition rates, STEAM is at least 1000 times faster than the state-of-the-art CCD and CMOS image sensors (Figure 2). STEAM's ultrafast shutter speed (~10 ps) freezes any motion of cells in high-speed flow, and its record frame rate (more than 10M fps) enables high-throughput identification of a large heterogeneous population of cells at the unprecedented throughput of 100,000 cells/s. This throughput is equivalent to screening of 10 mL of blood within 15 minutes (possibly containing ~100 CTCs among 54 billion blood cells in a cancer patient's blood).

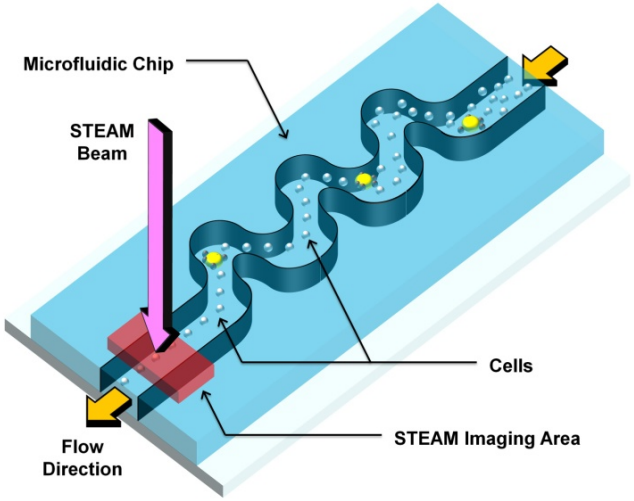

Figure 3: Microfluidic chip. In the microchannel, cells are focused and ordered into a single stream with inertial lift forces.

The other important components of STEAM automated image cytometry for real-time CTC detection are the microfluidic chip and real-time digital image processor. The microfluidic chip is based on the self-focusing microfluidic flow control technique known as inertial microfluidics [9, 10] such that cells in high-speed flow are focused into a single stream and hence can be imaged by STEAM without blur. This is important because out-of-focus blur causes misidentification of cell types in the image processing process. As for the digital image processor, it consists of a field-programmable gate array (FPGA) and central processing unit (CPU) for rough and fine screening, respectively (Figure 4). The FPGA enables real-time identification and classification of cells, reducing the computational burden on the CPU. State-of-the-art computational algorithms are implemented on the FPGA.

The integrated functionality of the instrument enables real-time detection of rare target cells in a large population of blood cells. Figure 4 shows the images of rare MCF7 breast cancer cells spiked in blood that were captured by the STEAM automated image cytometer with a throughput of 100,000 cells/s at a high capture efficiency of 75% (Figure 5) [4]. STEAM automated image cytometry is 100 times better in terms of false positive rate than conventional flow cytometry without sacrificing throughput and sensitivity (Figure 6) [4]. Its record low false positive rate enables detection of one cell in a million blood cells or one in a billion after lysis.

3. CONCLUSIONS

In conclusion, we have developed high-throughput automated image cytometry (known as STEAM automated image cytometry) for blur-free image acquisition and non-stop real-time image-recording and classification of cells in high-speed flow. This method holds promise for early, noninvasive, low-cost detection of cancer and evaluation of chemotherapy. While this

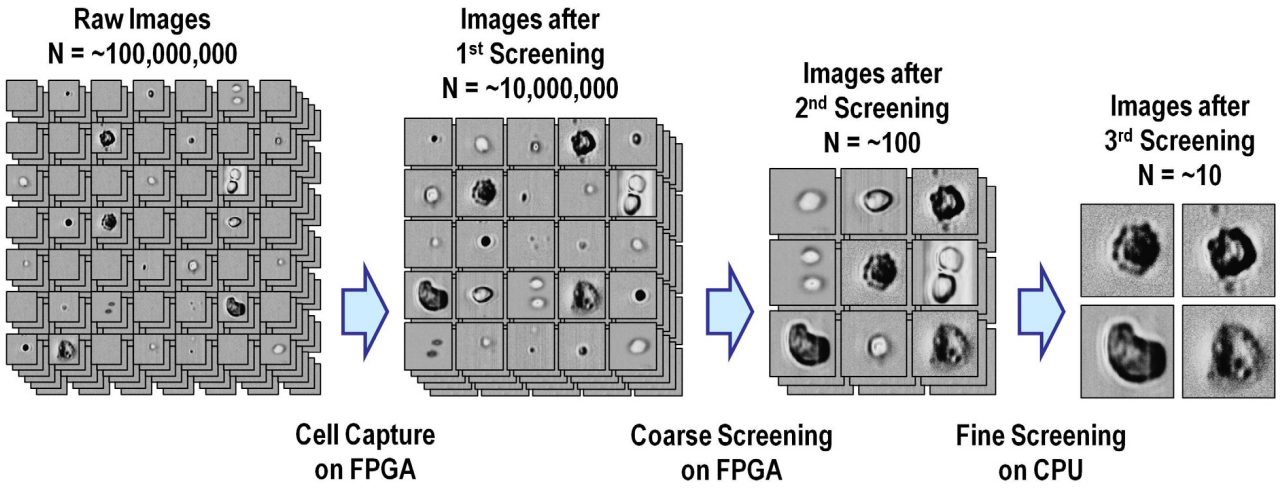

Figure 4: Multi-step screening process for identifying MCF7 breast cancer cells in a large heterogeneous population of blood cells. The FPGA performs object identification within the field of view and coarse screening by size while the CPU performs fine screening by presence of surface antigens and circularity.

method has been used to demonstrate detection of rare cancer cells in blood, it is also amendable to real-time identification of other types of rare cells such as antigen-specific T cells, fetal cells in maternal blood, and hematopoietic stem cells, provided that there are proper contrast agents are available.

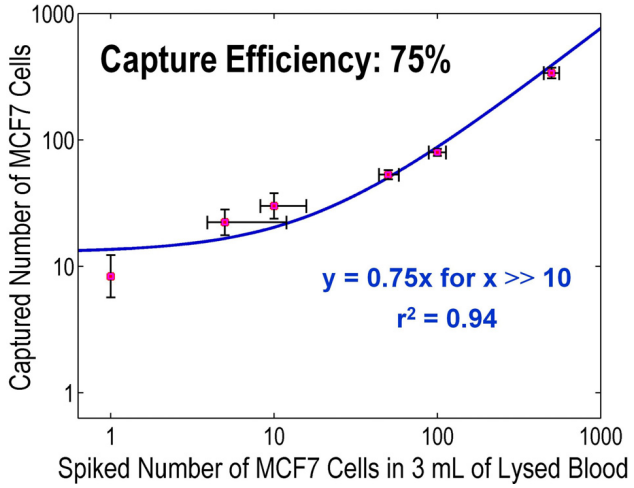

Figure 5: Capture efficiency of the STEAM automated image cytometer. The high capture efficiency is made possible without sacrificing the throughput. Here the capture efficiency of 75% is demonstrated with a high throughput of 100,000 cells/s.

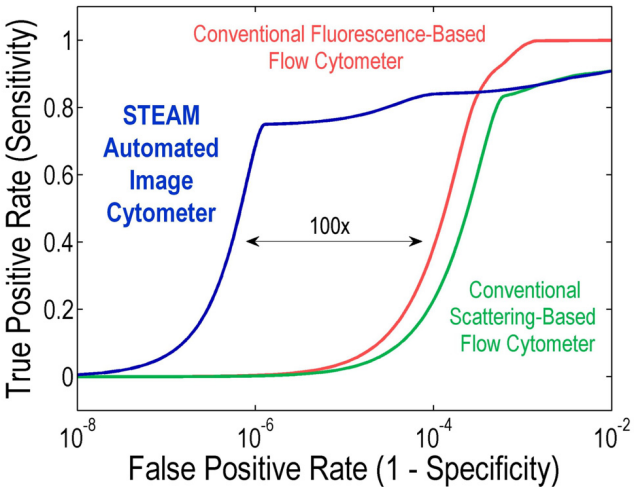

Figure 6: Receiver operating characteristic curves. The STEAM automated image cytometer is 100 times better in terms of false positive rate than conventional flow cytometry (both scattering- and fluorescence-based), yet with high throughput and high sensitivity.

4. ACKNOWLEDGEMENTS

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