COLOR SUB-PIXEL RESOLVING OPTOFLUIDIC MICROSCOPE AND ITS APPLICATION TO BLOOD CELL IMAGING FOR MALARIA DIAGNOSIS
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ABSTRACT
We report on the implementation of color sub-pixel resolving optofluidic microscope (SROFM), a high resolution (HR) on-chip imaging system for diagnostic applications in rural regions. With the combination of microfluidics and inexpensive complementary metal oxide semiconductor (CMOS) image sensors, we demonstrate optical resolution of 0.66 µm at the highest acuity. We applied the prototypes to perform color imaging of red blood cells (RBCs) infected with Plasmodium falciparum (P. falciparum), a particularly harmful type of malaria parasites and one of the major causes of death in the developing world.

KEYWORDS: Optofluidic microscope, Blood cell imaging, lensless imaging, Plasmodium Falciparum

INTRODUCTION
Miniaturization of imaging systems can significantly benefit clinical diagnosis in challenging environments, where access to physicians and good equipment can be limited. The optofluidic microscope (OFM) scheme offers lensless imaging in the form of an on-chip device that is manufacturable through semiconductor foundries. For many blood-borne diseases including malaria, the gold standard of diagnosis is with optical microscopy, where a large number of blood cells needs to be imaged with high optical resolution. SROFM offers high-throughput lensless imaging by combining an inexpensive CMOS sensor and a microfluidic channel. The scheme achieves HR imaging by scanning the specimens while taking a sequence of low-resolution (LR) direct-projection images, which are combined with pixel super-resolution algorithm to achieve resolution enhancement. SROFM combines the high sensitivity of microscopic diagnosis and the accessibility and low-cost of a portable device, offering a good alternative for the field diagnosis of malaria. Here we report on color imaging of blood samples with a SROFM device using color illumination for a proof-of-concept on-chip device for imaging-based malaria detection.

EXPERIMENTAL
Our prototype color SROFM system is depicted in Fig. 1A. We used sequential red-green-blue illumination to obtain a LR sequence for each color and then combine them to a single HR color image. Three light emitting diodes (LED) with wavelengths of 625 nm, 525 nm and 475 nm were used for illumination with a switching rate of 8Hz. Fig 1B shows the circuit board for the sensor and a SROFM chip, which consisted of a CMOS image sensor and a poly-dimethylsiloxane (PDMS) microfluidic channel mounted on top (Fig 1C), with a total size of approximately 1 cm by 1 cm. The pixel size of CMOS sensor used in all experiments was 2.2 µm.

![Figure 1](image_url)

Sample was flown through the microfluidic channel and the sensor takes a sequence of direct projection images from underneath the channel. The resolution of these direct projection images limited by the pixel size of the sensor. The sub-pixel resolving algorithm shifts each LR frame by the relative sub-pixel shift of the sample and adds all the frames all together to fill a HR image grid with the enhancement factor (EF) of 10. The motion vector of each cell, a vector which describes the sub-pixel shift of each frame for rearrangement of each frame in the high resolution matrix, was obtained by tracing the movement of each blood cell in the raw sequence by custom particle tracking software. In order to scan the sample through
two dimensions, we aligned the PDMS microfluidic channel with a small angle (7.5°) with respect to the pixel grid. We designed the microfluidic channel with a height of 5.5 µm so that the blood cells were translated near the surface of the active area of the sensor without rolling. In order to reduce in-plane rotation of the cells, inertial focusing of the blood cells was introduced by putting obstacles along the walls in the beginning part of the channel.

Figure 2. (a) Conventional bright field microscope image of the same field of view, taken with a 10x objective lens. (b) Blood cells in a SROFM device directly taken from the CMOS image sensor. (c) LR sequence of a single RBC to a HR image converted with EF = 10. (d) Human blood cell images taken with SROFM. Scale bars indicate 20 µm in (a),(b) and 5 µm in (c),(d).

RESULTS AND DISCUSSION

The optical resolution of the device was investigated by imaging 500 nm microspheres with our SROFM device. We used 500 nm blue-dyed microspheres to enhance the contrast of the microspheres images. The microspheres were flown through a 1.5 µm-thick channel. As shown in fig. 3A, the bright centers of the microspheres were clearly resolved, with the full-width half maximum (FWHM) of 590 nm under EF of 10, suggesting the optical resolution of the device was 660 nm since two spots would need to be at least 3 high resolution pixels apart to be distinguished.

To verify the color capability of the RGB illumination method with SROFM, we measured the light transmission through the microfluidic channel with different concentrations of Trypan blue dye. According to Beer-Lambert law, we expect \(-\ln(T/T_0)\) to increase linearly under increasing concentration of dye, with the slope corresponding to the absorption cross-section \(\sigma\) for each illumination wavelength. Fig. 3B confirms that the logarithm of signal transmission through Trypan blue dye is proportional to the concentration of the dye and that the relative values of \(\sigma\) for the three wavelengths agree with the known absorption spectrum of Trypan blue dye.

For imaging of blood cells, a blood sample was diluted to 1:50 with Phosphate buffered saline (PBS), in order to prevent overlapping of the cells in the channel and heparin was added to a final concentration of 100 U/ml to prevent clogging. The pre-
treated whole blood sample was injected into the inlet of the device and flown through the channel. As the blood cells flowed across the channel, low resolution images were obtained from the image sensor with a region of interest (ROI) of 300 by 80 pixels at the frame rate of 800 fps. With the current values of sample flow rate and low resolution imaging frame rate, the system can scan approximately 400 cells/sec for monochromatic imaging and 100 cells/sec for color imaging.

To demonstrate the potential application of our color SROFM device for malaria field diagnostics, we imaged RBCs infected with *P. falciparum*. We used Toluidine blue dye to stain the parasites in solution, which does not require fixation of cells and thus simplifies the sample preparation protocol, as opposed to Giemsa staining for typical blood smears. In this experiment, we purified cultures of *P. falciparum* to obtain infected RBCs (iRBCs) in the schizont or late trophozoite stage at a concentration >98%. Samples were treated with 2% Toluidine blue, to stain the *P. falciparum* parasites within. Color SROFM images of *P. falciparum* iRBCs at the schizont-stage and naïve RBCs show clear differences between the two stages, with the bright purple spots in the iRBCs indicating the presence of parasites. Our preliminary experiment indicates that color SROFM can potentially be a useful diagnostic tool for identifying malaria infected cells.

![Figure 4](image_url)

**Figure 4.** (a) Color images of *P. falciparum* schizont-stage RBCs stained with Toluidine blue, showing a distinct purple spot within each RBC, which appears as dark spots in the green channel. (b) Color images of naïve RBCs show clear differences with the infected RBCs. Naïve RBCs were also stained with Toluidine blue as a control.

CONCLUSION
We have developed a compact high-resolution color imaging microscopy device based on sub-pixel resolving optofluidic microscopy (SROFM) and color illumination. Our device achieved an optical resolution of 660 nm. Our preliminary experiment indicates that color SROFM can potentially be a useful diagnostic tool for identifying malaria infected cells.

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REFERENCES
[1] Cui X, Lee L, Heng X, Zhong W, Sternberg P, et al. "Lensless high-resolution on-chip optofluidic microscopes for Caenorhabditis elegans and cell imaging", Proc. Nat'l. Acad. Sci., vol. 105, pp.10670, 2008.
[2] Zheng G, Lee SA, Yang S, Yang C, "Sub-pixel resolving optofluidic microscope for on-chip cell imaging", Lab. Chip., vol. 10, pp. 3125-3129, 2010.
[3] Bishara W, Su T, Coskun A, Ozcan A, "Lensfree on-chip microscopy over a wide field-of-view using pixel super-resolution", Opt. Express, vol 18, pp. 11181-11191, 2010.
[4] Breslauer D, Maamari R, Switz N, Lam W, Fletcher D, "Mobile Phone Based Clinical Microscopy for Global Health Applications", PLoS ONE, vol 7, pp. 6320, 2009.

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