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LED excitation of an on-chip imaging flow cytometer for bead-based immunoassay

Xilong Yuan, Todd Darcie, J Stewart Aitchison
Department of Electrical and Computer Engineering
University of Toronto
Toronto, M5S 3G4 Canada

Jonathan J.D. McKendry, Michael Strain, Martin D. Dawson
Institute of Photonics, SUPA, Department of Physics
University of Strathlcyde
Glasgow G1 1RD United Kingdom

***Abstract*— A green LED is demonstrated to generate a uniform square illumination pattern for an on-chip imaging flow cytometer system. The proposed system is used to perform the detection of a bead-based immunoassay for a sepsis biomarker, procalcitonin.**

Keywords—LEDs, imaging, immunoassay, fluorescence, flow cytometer, biomarker detection

I. Introduction

In the context of globalization and climate change, effective management of the outbreak of infectious diseases is an essential but also challenging task for policy makers, clinicians and scientists. One of the many unmet needs is the rapid triage and diagnosis of patient conditions according to symptoms and test results.[1, 2] For example, a typical naso-pharynx swab pathogen testing will have following steps: sampling in an access center, transferring to a central laboratory, performing biology testing manually or automatically on analytical systems, and reporting results to central laboratory/medical information systems. This workflow essentially relies on the personnel, facility and instruments in central laboratories. Therefore, there are emerging needs to build a scheme of decentralized laboratory tests, such as a pathogen screening device in a community healthcare hub, a blood biomarker profiler at intensive care units, etc.[3, 4]

Bead-based immunoassay is one type of suspension array technology to detect the abundance of protein biomarkers in a complex sample.[5] Polystyrene beads are typically utilized as the substrate for binding with target molecules with high specificity. Because polystyrene beads can be pretreated with fluorescent dyes in varying combinations of dye type and concentration, a panel of beads can be designed for simultaneous detection of multiple biomarkers with high sensitivity.[5] Typically, a flow cytometer is used to measure the fluorescence intensity of beads. In our previous research, lasers were used for the illumination of an imaging system.[6] It is difficult to generate a uniform top-hat laser beam across the microfluidic channel plane. However, fluorescence excitation does not require a coherent light source. Therefore, LEDs are considered an attractive alternative to laser excitation. With the development of GaN LEDs over the last 20-30 years, LEDs are now available at wavelengths that cover from the deep ultraviolet to the near infrared, providing flexibility in matching suitable LEDs with the requirements of different fluorophores. Lower risk to eyes is another benefit of LEDs since they are inherently less dangerous than lasers. Additionally, increased LED brightness can be achieved, and LEDs can be powered by low-voltage batteries or relatively inexpensive switchable power supplies.

In this paper, we proposed and experimentally demonstrated the fluorescence detection of a bead-based immunoassay in a microfluidic channel. Compared to the laser source typically used in a conventional flow cytometer, this system is comprised of LEDs as the light source of a potential portable diagnostic device. Additionally, we adopted a wide and shallow microfluidic channel for the on-chip imaging device, allowing more bead images captured in a specific field of view.

II. LED and the Imaging System

The bead-based immunoassay can identify the type of biomarker in a 'bead set' by decoding the intensity of the 'barcode fluorescence' (Fig. 1A). The intensity of detection fluorescence is correlated with the concentration of a specific biomarker. Allophycocyanin (APC) is the barcode fluorescent dye, and phycoerythrin (PE) is the detection fluorescent dye. An excitation bandpass filter was used to block the wavelengths that may overlap with the fluorescence emission bands, as shown in Fig. 1B.

As shown in Fig. 2, two identical aspherical lenses were used to image the LED light on the plane of a microfluidic channel. A green LED (Osram LE RTDUW S2WP) with 520 nm peak wavelength is selected to excite the two fluorescence dyes (APC and PE). The 1 mm x 1 mm LED can illuminate the entire width

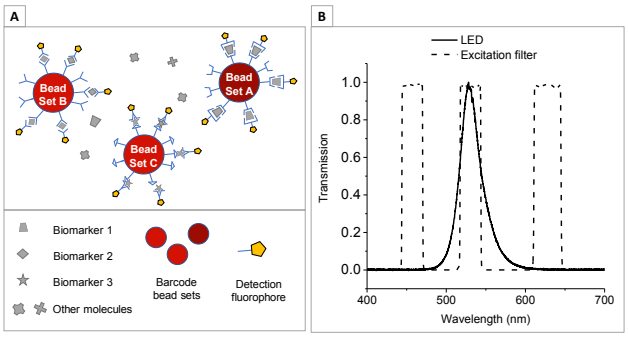

Figure 1. A) Schematic of bead-based immunoassay. B) Spectra of the green LED and the excitation filter

of the microfluidic detection channel (800 µm) with an estimated optical power of 16 mW/mm^2.

The imaging module is comprised of an infinity-corrected optical system. A dual bandpass filter (center wavelength 577 nm and 690 nm) is used to transmit the emission fluorescence from the two fluorescent dyes. Additionally, a bandpass filter with 565 nm center wavelength and a longpass filter with 665 nm cut-on wavelength are assembled on flip mounts, allowing for versatile filter swapping. To take an image of the intensity profile of the LED light on the microfluidic channel, neutral density filters (OD 5.0) are applied. The LED intensity profile across the microfluidic channel has a percentage variation of 12.7% and 7.2% for x axis and y axis respectively. It is considered satisfactory for further testing of fluorescence emission.

III. Fluorescence Imaging and Biomarker Detection

To characterize the performance of LED illumination for the on-chip imaging flow cytometer, a custom developed bead-based immunoassay of procalcitonin is performed. Procalcitonin is a sepsis biomarker that has clinical significance for diagnosing the severity of infectious conditions such as sepsis. A cut-off concentration lower than 0.5 ng/mL is indicative of absence of infection or a mild infection. In patients with severe sepsis

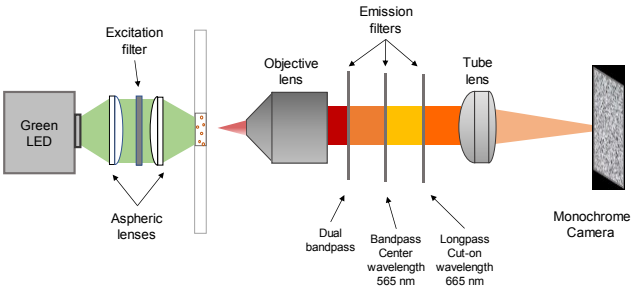

Figure 2. Schematic of the on-chip imaging cytometer using LED as the excitation source.

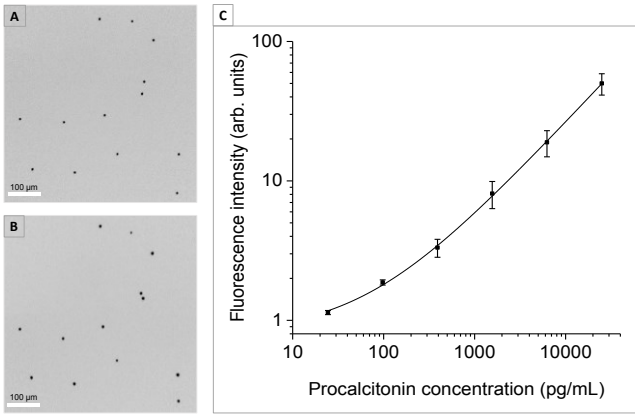

Figure 3. A) Inverted image of beads with FGL-665 longpass filter (thorlabs) showing the fluorescence of APC. B) Inverted image of beads (procalcitonin concentration is 0.156 ng/mL) with MF-565/24 bandpass filter, showing the PE fluorescence. C) Calibration curve of the procalcitonin concentration against the median detection fluorescence intensity of the beads. Error bars showed fluorescence intensity variations in multiple capture images (n>=3).

complications and less likelihood of survival, procalcitonin levels could reach 10 ng/mL or higher.[7]

Fluorescence beads containing a pre-defined level of APC fluorescence are used because it permits multiplex detection of multiple biomarkers in future developments. As shown in Figure 3A, the barcode fluorescent dye APC can be detected by applying a longpass filter. Sandwich immunoassay of procalcitonin is performed by incubating varying concentrations of procalcitonin standards (6.1 pg/mL up to 25 ng/mL) with beads containing a procalcitonin-specific capture antibody. Fig. 3B shows a image of beads after applying a bandpass filter at center wavelength of 565 nm. This fluorescent band corresponds to the fluorescence of PE, the reporter dye used in the fluorescence immunoassay.

To plot a calibration curve of fluorescence intensity against procalcitonin concentration, multiple images of beads were captured for each immunoassay sample. Integrated intensity of the 'bead event' are used for fluorescence intensity evaluation. At least 20 bead events were used for statistical analysis. Events are randomly arranged into groups (n>5) and then median fluorescence intensity is calculated for each group. As shown in Fig. 3C, fluorescence intensity is fitted with procalcitonin concentration using logistic regression statistics. Parallel testing of beads using a conventional flow cytometer found that flow cytometer could detect lower fluorescence signal intensity, translating to a procalcitonin concentration of 6.1 pg/mL. The proposed LED on-chip imaging flow cytometer can detect the procalcitonin concentration of 24.4 pg/mL. Both concentration levels are below the cut-off concentration of procalcitonin for sepsis diagnosis, indicating the potential of the proposed system for point-of-care testing.

IV. Conclusive Remarks

The proposed LED illumination and on-chip imaging system is capable of detecting protein biomarkers with high sensitivity. Future work may focus on characterizing the multiplexing capability of the system, fabricating the device in a compact prototype and investigating the sample preparation module of the system.

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