MULTI-SPECTRAL FLUORESCENCE MICROSCOPY WITH EMBEDDED LIQUID FILTERS FOR POINT-OF-CARE APPLICATIONS

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ABSTRACT

We present a new way of multi-spectral fluorescence microscopy using the embedded liquid filters in a microfluidic chip, providing flexibility of filter changes for target fluorescence wavelengths and low-cost assays for point-of-care (POC) applications. Widely available food color solutions are adopted for emission filter to absorb residual excitation light. Absorbance of the microfluidic liquid filter was measured for various dye concentrations and filter layer thicknesses. Also, absorbance spectrum of two different color mixtures was investigated for tuning the filter wavelength. Both results match with theoretical Beer-Lambert Law. On-chip liquid-filter fluorescence imaging was demonstrated with multiple LED light sources and fluorophores. Live (green)/dead (red) fluorescence images of C6 cells viability assays were successfully demonstrated using the fabricated microchip with the embedded liquid filters. The images taken with the liquid filter were comparable to those from the conventional microscope using FITC HYQ and TRITC HYQ filter sets.

KEYWORDS: fluorescence microscopy, liquid filter, POC, optical microfluidics, contact imaging

INTRODUCTION

Fluorescence microscopy, especially benefiting from vast number of fluorophores, provides powerful visualization of tissues, cells, subcellular structures, and specific biomolecules tracking and analyses [1]. However, traditional fluorescence microscopy limits its applications within the central laboratory setting and hospitals due to high cost, bulkiness and complicated setups. Integration of microfluidics and fluorescence imaging technologies can provide an ideal solution for patient diagnostics at low cost [2-4]. However, the previous approaches are limited by the fixed solid filters that can only work for a specific spectrum and is difficult to switch from one filter to another, once they are integrated within a system such as contact imaging diagnoses. Up to date, microfluidic liquid filters have been utilized for gray-scale photolithography or exposure dose control of high-throughput PDT drug screening [5, 6]. In this work, we report multi-spectral fluorescence imaging in a microfluidic chip with the embedded refreshable liquid filters that utilize food color dyes. Liquid filters, as a homogeneous component of microfluidic systems, provide comparable performance to the conventional solid filters and easily customize the absorption wavelength by flowing a combination of different dyes.

DEVICE DESIGN

As shown in Figure 1, the proposed chip is composed of two PDMS layers: top fluorescent sample layer and bottom liquid filter layer, separated by an 80 μm-thick glass substrate. Fluorophores are excited by an LED light source, which has an incident angle of 75°. Fluorescence emission is collected by the lens beneath the chip and captured by a CCD sensor, while the scattered excitation light and other undesirable emissions are blocked by the liquid filter. Figure 1(a) and (b) show the cross-sectional view of the imaging system setup. LED light source and/or liquid filter can be switched according to the target fluorescence imaging. Most excitation light from the LED passes through the chip and won't be collected by the lens due to a large incident angle, and any scattered light will be blocked by the liquid filter.

EXPERIMENTAL AND RESULTS

Three different types of commercial food dyes have been used: tartrazine (FD&C yellow 5), allura red (FD&C red 40) and brilliant blue FCF (FD&C blue 1) (McCormick). Absorbance spectra of color dyes and their mixtures were measured with a spectrophotometer (Agilent, model 8341) after diluted

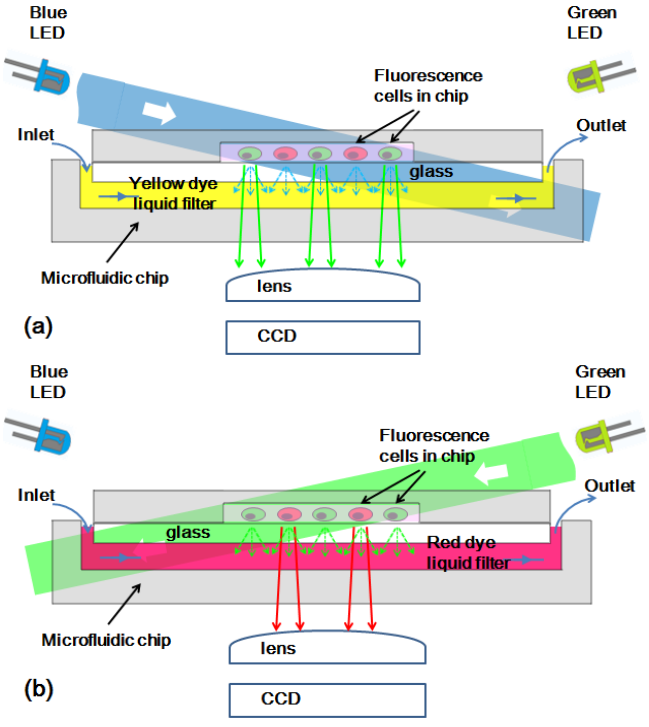

Figure1. Cross-sectional views of the imaging system taking green (a) and red(b) fluorescent images.

with DI water to 10^3ppm. Figure 2 shows the measured absorbance spectra of color dyes and their mixture in the wavelength between 300nm and 800nm. Yellow, red and blue dye solutions had an absorbance peak at the wavelength around 427nm, 498nm and 630nm, respectively, and their mixtures showed the summation of absorbance for its individual ingredients. This means we can simply add a corresponding dye to the liquid filter solution when we want to block an additional wavelength.

A blue LED (470nm) and yellow dye solution were used to characterize the absorbance under different dye concentrations and liquid filter thicknesses. We used a Nikon TE2000-U inverted microscope with a 20x objective to measure the light intensity at the surface after passing through the liquid filter. The images were recorded using Coolsnap ES Monochrome Camera with MetaVue software. First, images were taken with the liquid filter filled with DI water (no absorption) and yellow dye solution ranging from 10^4ppm to 10^5ppm. An image taken with LED light source off was used as control background. All the images were taken with an exposure time of 10ms and sampled at the same position with an area of 100 by 100 pixels. ImageJ software was used for image processing (subtracting background). Absorbance was calculated from the equation, $A = -\log_{10}(I/I_0)$, and plotted in Figure 3. Absorbance increases linearly with dye concentrations and light path lengths (filter channel heights), which well agrees with Beer-Lambert law.

Two fluorophores, Fluorescein isothiocyanate (FITC) and pyranine solutions, were used for demonstrating the potential use of the liquid filter in the application of microfluidic multi-spectral fluorescence ELISA-like assays and immunoblotting. In this experiment, neighboring microfluidic channels for cell assays (90μm in height) were filled with 10^3ppm FITC and 10^3ppm pyranine, respectively, in the top layer. Two light sources were implemented: One of them is a 470nm blue LED for FITC excitation and the other is a 380nm UV LED for pyranine excitation. Liquid filter layer was filled with 10^5ppm yellow color, which has a similar high absorption coefficient at both LED wavelengths but has negligible absorption in both fluorophores (FITC and pyranine) emission wavelengths. Images were recorded and processed in the same way as described above. Figure 4(a) shows the emission from FITC (channel 1, blue LED on), Fig. 4(b) from pyranine (channel 2, UV LED on), and Fig. 4(c) from both channels (both LEDs on). We could clearly distinguish these two fluorophores by switching the light sources.

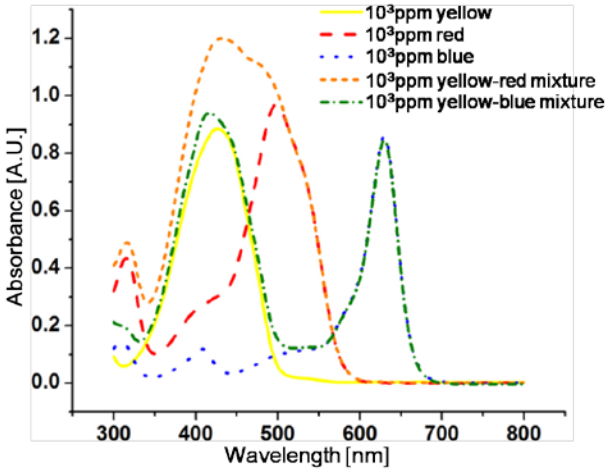

Figure2. Absorbance spectra of different food dyes and their mixtures.

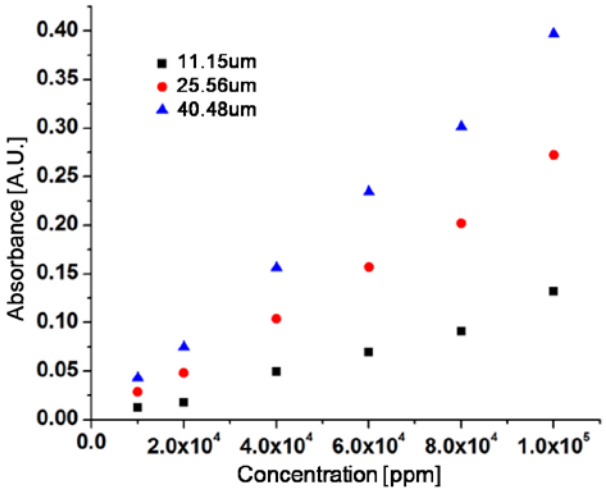

Figure3. Absorbance of yellow food-dye color filter illuminated by a blue LED light source for various channel thicknesses and concentrations from 10^4ppm to 10^5ppm in semi-log scale.

Multi-spectral fluorescence imaging of real cell samples was demonstrated using C6 glioma tumor cells. C6 cells in culture media (DMEM+5%FBS+0.5%penicillin) were loaded into the top sample layer and cultured for 6 hours until they were fully attached on the glass substrate. Then, in the half of channels, culture media were replaced with 70% ethanol and kept for 30mins to kill the cells. After that, all channels are refreshed with Calcein AM (10uM, live, green) and Ethidium homodimer-1 (10uM, dead, red) in DMEM for live/dead fluorescence staining. Fluorescence images taken from the fabricated liquid filters were captured and compared with those taken from the conventional microscope filter sets. Figure 5(a) shows the image acquired by the conventional microscope using FITC HYQ and TRITC HYQ filter sets, with a X-Cite 120 illumination system light source. In order to capture the images from the liquid filter, a 473nm blue laser light source was used for excitation of both fluorophores. Figure 5(b) shows the image acquired using the integrated liquid filter. For green cells, we used a mixture of 10^5ppm yellow and10^5ppm blue dyes. For red cells, we used a mixture of 10^5ppm yellow and 10^5ppm red dyes. Figure 5(c) is the enhanced image after background subtraction using ImageJ, in which the spectral range other than the targeting fluorescence was further removed. All images were pseudo-colored and having color channels merged with ImageJ software.

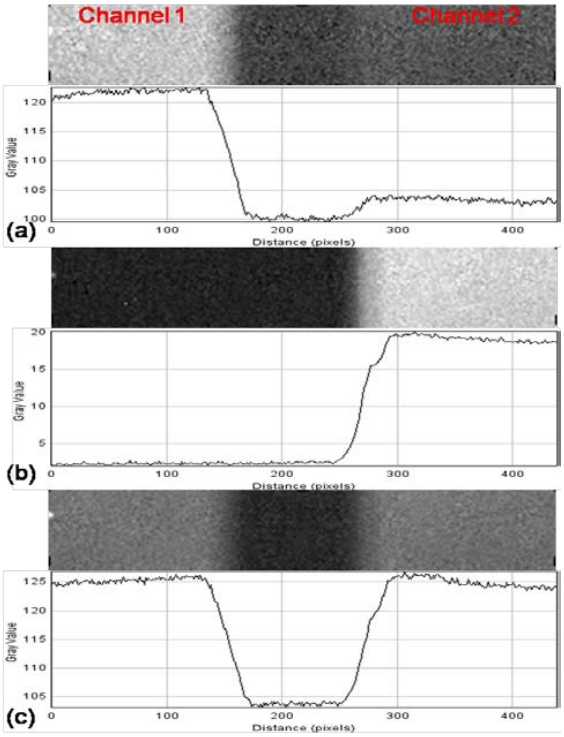

Figure4. Fluorescence imaging of FITC (channel 1) and pyranine (channel 2) under different excitation conditions: (a) blue LED exposure, (b) UV LED exposure, and (c) both UV and blue LED exposure.

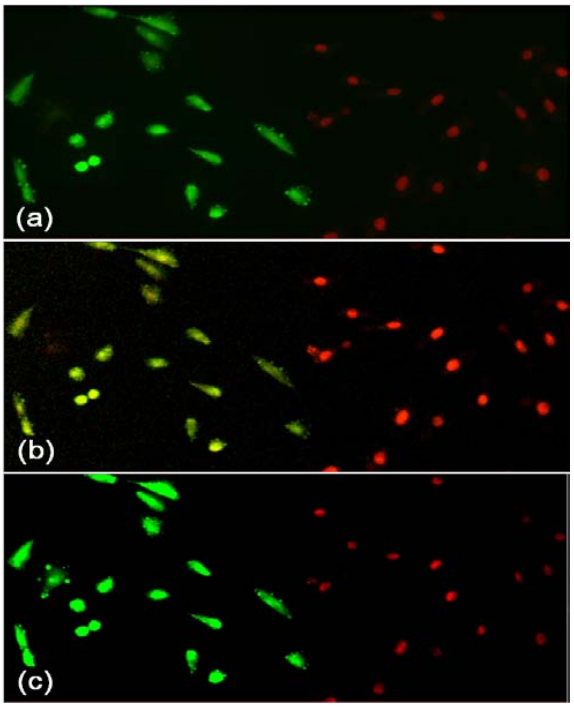

Figure5. (a) Live/dead stained C6 cells observed by the conventional microscope with FITC HYQ and TRITC HYQ filter sets, (b) raw image from the integrated liquid filter filled with food color dyes, and (c) the image of (b) taken after background subtraction using ImageJ.

CONCLUSION

We demonstrated the feasibility of multi-spectral fluorescent imaging using the embedded liquid filters in microfluidic chips. LED light sources and widely available food dyes provide a simple and low-cost imaging setup. Absorbance spectra of the liquid filters were characterized for various food dye concentrations and filter thicknesses, showing a good match with the Beer-Lambert law. Multi-spectral imaging for fluorescent solution and cell samples were also demonstrated and showed the comparable results to the conventional imaging using microscope filter sets. Due to its low-cost, compact-integration and diverse fluorescence detection capability, the proposed fluorescent imaging device can find good niche applications in lab-on-a-chip platforms and POC diagnostic sensors.

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