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# High-sensitivity integrated fluorescence analysis for microfluidic lab-on-a-chip

**David Klotzkin and Ian Papautsky**

*A microdevice equipped with polarizing filters and an organic photodiode enables detection of dye concentrations in the low nanomolar range.*

Microfluidics studies the behavior of fluids at the microscale and the design of systems to take advantage of such behavior. A multidisciplinary field encompassing physics, chemistry, engineering, and biotechnology, microfluidics integrates sensors, actuators, and other electronics to create new applications.[1–3] Importantly, new principles of fluid manipulation have enabled detection and handling of nanoliter fluid samples. In recent years, these principles have been applied to the development of lab-on-a-chip (LOC) systems.[4,5]

A substantial challenge for such miniaturized systems—the size of a credit card—for point-of-care and on-site analysis lies in developing their ability to effectively detect analytes of interest. Small sample volumes and low concentrations common to microfluidic LOCs make high-sensitivity detection of critical importance. While conventional microscopy tools can easily meet these demands in a laboratory, developing portable systems requires integrated miniaturized detectors.

Fluorescence is one of the most commonly used analytic techniques in the biosciences. It is based on emission of a dye at one wavelength when it is illuminated by another, shorter wavelength. Lack of integrated fluorescence detection is a major roadblock for many biotechnological assays in portable LOC format.

In a typical microfluidic fluorescence immunoassay assay, fluorescently labeled antibodies are used to tag a specific antigen (e.g., bacteria, viruses, or other organic molecules of interest) in a microfluidic device. The excitation light stimulates the dye to fluoresce, and the characteristic emission wavelength, or color, is observed through a filter that suppresses excitation light, enabling the dye emission to be seen clearly. As shown in Figure 1, the orange light represents the dye emission and the green light is the excitation signal. As suggested by the relative size of the arrows, the excitation light is orders of magnitude higher in intensity than the emission light from the dye.

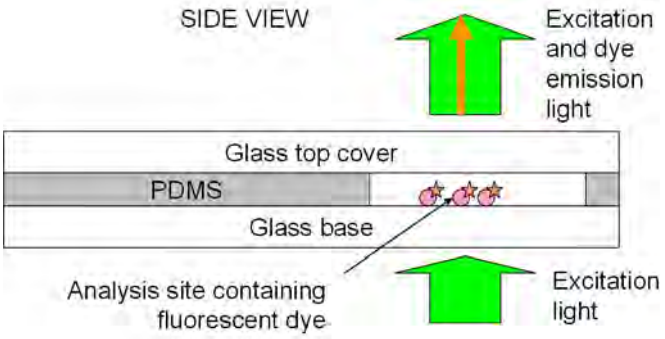


***Figure 1.*** *Schematic of a lab-on-a-chip (LOC) for fluorescence analysis. PDMS: Poly(dimethylsiloxane).*

The challenge in moving from a conventional setup (see Figure 2) to a credit-card-sized system is incorporation of the filter. Light sources and detectors made with easily deposited organic materials can be readily fabricated on glass and physically integrated into the LOC.[6] In the sandwich configuration shown in Figure 1, the detector will not give information about the dye concentration, as the detector emission signal from the dye is typically overwhelmed by the excitation light. Various approaches to overcoming this problem have been proposed, including an integrated wavelength filter[7,8] and a directional solution, by which the excitation light is highly directional and the detector oriented such that it only sees the isotropically emitted dye light.[9]

Our simple and general integrated solution uses polarizers to isolate the excitation light from the detector, as indicated in Figure 3. The excitation light is polarized, but the fluorescence

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from the dye is emitted with random polarization. A second polarizer, oriented at 90° to the first, is placed in front of the detector. Because the excitation light must cross two polarizers, its intensity is reduced by ∼30dB.

This fairly elegant solution works with any combination of excitation and emission light, even if the two signals overlap in wavelength. Figure 4 shows representative results of a quantitative analysis made using a filtered halide light source for excitation, an organic photodiode made with CuPC/C60 (copper phthalocyanine/fullerene),[10] and a microfluidic mixer. The dye is Rhodamine 6G, which emits orange-red (585nm) and is excited by green (510nm) light. The intensity signal from the organic photodiode is proportional to concentration down to 10nM. This represents a 1000-fold improvement in sensitivity from the best previous reports.[11] Also shown is an analysis without polarization filtering, indicating much higher signals that are not proportional to the dye concentration. The analysis with polarizers (illustrating the orange dye light) and without polarizers is shown in Figure 3, with the bright green excitation light swamping the dye signal.

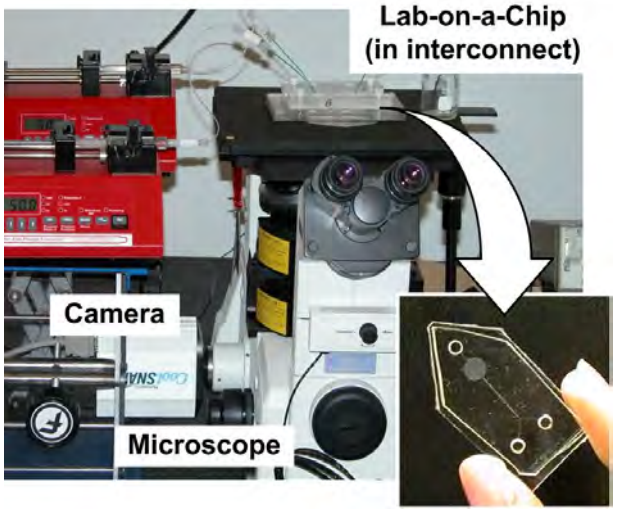


***Figure 2.*** *A typical microscope setup for microfluidic fluorescence analysis to be miniaturized into an integrated LOC (inset).*

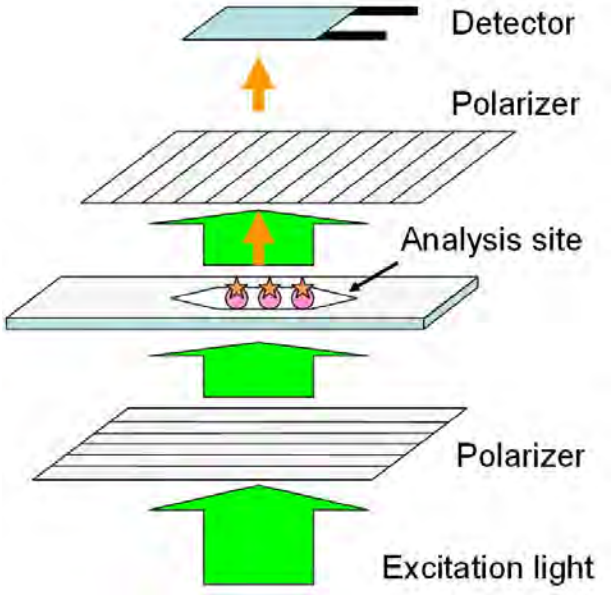


***Figure 3.*** *Schematic of the polarization filtering analysis system.*

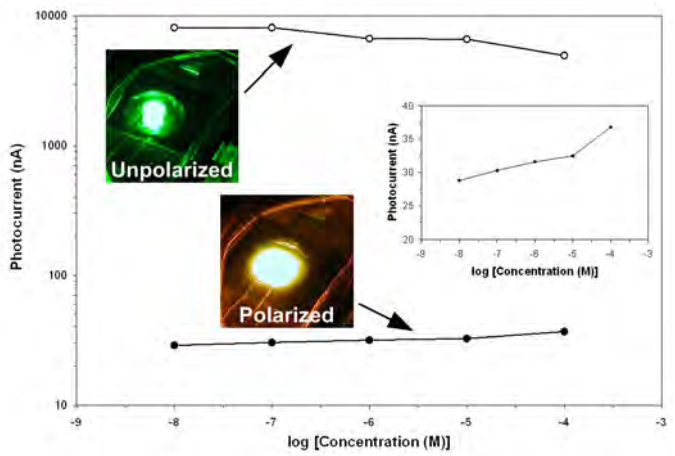


***Figure 4.*** *Quantitative analysis with the polarization filtering system, illustrating detection sensitivity down to 10nM.*

This system works well for two reasons. The crossed polarizers by themselves provide excellent isolation, and spectral characteristics of the organic detector have much higher responsivity in red than in green, providing still more isolation. The device shown here demonstrates proof of principle with external components. But it can be readily fabricated as an integrated device with organic detectors, photodiodes, and microfluidic mixers all on the same glass substrate. This is a practical technology for realizing integrated fluorescence analysis microfluidic devices, and it represents a significant step toward complete microfluidic LOCs for point-of-care and on-site analysis.

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## Author Information

**David Klotzkin**
Optoelectronics Device Laboratory
Department of Electrical and Computer Engineering
University of Cincinnati
Cincinnati, OH
http://www.ece.uc.edu/∼klotzkdj

**Ian Papautsky**
BioMicroSystems Laboratory
Department of Electrical and Computer Engineering
University of Cincinnati
Cincinnati, OH
www.biomicro.uc.edu

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