

Improving fluorescence detection in lab on chip devices

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Introduction

Fluorescence is the workhorse for analysis in many of today's lab-on-chip (LOC) devices.¹ However, LOC fluorescence detection is limited in both spatial resolution and sensitivity, due primarily to sub-optimal light collection. More sensitive, higher resolution LOC devices would enable novel structural and functional biomolecule studies, as well as improve chip-based bioanalytical assays by orders of magnitude. In this focus article, we consider the possible avenues for performing high-sensitivity and high-resolution fluorescence detection within LOC devices.

Fluorescence detection as a workhorse

Today's LOC analytical devices use a variety of detection platforms, including electrochemical detection,^{2,3} UV absorbance,^{2,4} contactless conductivity detection,⁵ amperometry,⁵ Raman detection,⁶ infrared detection,⁷ nuclear magnetic resonance (NMR),^{8,9} photoacoustic detection¹⁰ and surface plasmon resonance (SPR).¹¹ Other detection techniques are constantly being developed.^{12–14} However, among the plethora of LOC detection platforms, fluorescence is the workhorse—the most inexpensive and the most widely used.

Fluorescence detection has many advantages. It uses small, well-characterized and generally non-perturbative labels that are amenable to conventional linking chemistries and available in a wide range of colors for multiplexing (Fig. 1). The latter means different analytes can be identified and distinguished on-chip not only by position, but also by spectra. With careful choice of label placement and spectral properties, fluorescence can report on nm-scale changes in molecular structure *via* mechanisms such as Förster resonant energy transfer (FRET), a distance-dependent interaction between two fluorophores¹⁵ Fluorescence can also provide a measurement of conformational dynamics¹⁶ and molecular function, like

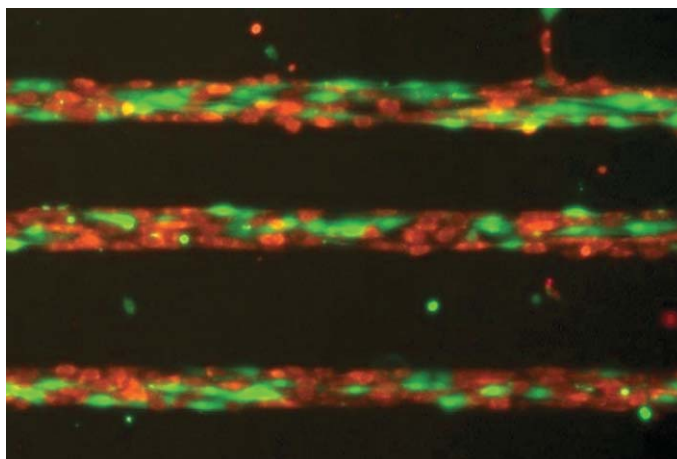


Fig. 1 Micropatterned substrates to study cell differentiation. Shown are muscle cells adhering to lines of Matrigel, a basal lamina extract, separated by non-adhesive strips of a poly(ethylene glycol) graft on glass. Two cell populations are separately labeled with live cell dyes and are mixed and seeded simultaneously on the micropatterned substrates. In the span of a few days, the red and green muscle cells in each track will fuse into a single cell, a multi-nucleated "myotube" that will appear yellow (the sum of red and green in the light spectrum). The cells left behind from the differentiation process will not be part of the myotube and will still be red or green. Such fluorescence assays in precise geometrical arrangements can offer insights into cell differentiation. (Courtesy Professor Albert Folch, University of Washington, USA.)

catalytic activity or binding affinity.¹⁷ Finally, high-resolution fluorescence imaging can reveal structural qualities of macromolecular polymers such as stiffness, shape, and entanglement.^{18–20}

Present state of the art

LOC devices are typically fabricated out of glass or PDMS and connected to a fluid handling system by microfluidic fittings in a chip holder with a cut-out that allows optical access for long working-distance objectives. Fluorescence in LOC devices is usually observed using either an upright or inverted microscope setup. With an inverted microscope setup, the objective is placed on a focusing mechanism below the sample, allowing stable support and easy access from above. Light, usually from a laser or an arc lamp, is directed through a microscope objective to the region of interest within the LOC device. The light is restricted to a narrow range of wavelengths that can both excite the fluorophores and be strongly excluded

from the detection channel. When not intrinsic to the light source, the narrow wavelength range is imposed by one or more interference filters and a dichroic mirror. Light emitted by the fluorophore is collected by the same microscope objective and sent to a detector, passing through the dichroic and (an)other filter(s) that exclude the excitation light. Common detectors include photomultiplier tubes (PMT) and avalanche photodiodes (APDs), which sense single photons with fast response times but provide no spatial information, and charged-couple device (CCD) arrays, which provide spatial information at the expense of sensitivity and/or longer integration times. A schematic of a typical setup for fluorescence detection is depicted in Fig. 2.

The need for more sensitive, higher resolution detection

One primary motivation for LOC devices is to minimize the amount of analyte required for analysis. Improved sensitivity

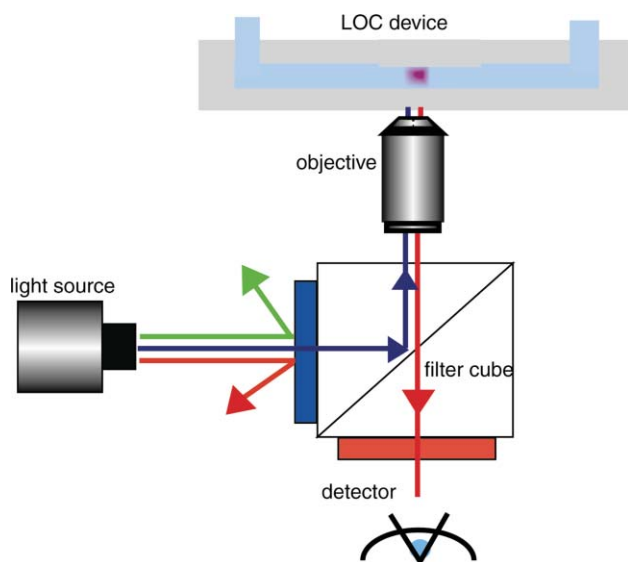


Fig. 2 Schematic of a typical setup for fluorescence detection from a LOC device. Note that the same lens is used to both illuminate and collect light from the sample. The numerical aperture of this lens is critical to the quality of the imaging but often limited by the geometry of the chip and its holder.

is therefore desirable in LOC detection platforms. For fluorescence in particular, greater sensitivity raises the prospect of high-resolution imaging and/or spectroscopy, which would extend the power of microfluidics to novel analyses. For example, high-resolution on-chip microscopy would allow real-time analysis of supramolecular assemblies in a more controlled 2D geometry and a wider variety of solution conditions than has been practical to date. Such studies would help elucidate the stability and kinetics of supramolecular assembly and advance the development of self-assembled devices.¹⁹ High-sensitivity, high-resolution imaging would also enable the use of quantum dots in particle image velocimetry (PIV). PIV measurements to date typically use 100–500 nm particles to trace velocity profiles in channel flows.²¹ With higher sensitivity, 6–20 nm quantum dots can be used instead, achieving an order of magnitude greater resolution of the velocity profile,^{22,23} and permitting the determination of flow fields in less accessible regions of 3D nano- and microchannel geometries, especially when the Peclet number is large.²⁴ Finally, by combining imaging and spectral resolution, on-chip fluorescence could greatly accelerate the development of multiplexed single molecule measurements for faster, more routine analysis of reaction rate distributions.²⁵

Prospects for the future

In this section, we consider several near-term and farther-term possibilities for increasing the sensitivity and resolution with which fluorescence is detected on-chip. We hope that this discussion stimulates readers to invent and disseminate technologies for higher performance fluorescence in LOC devices.

Near-term solutions

Significant improvement of fluorescence imaging in LOC devices can be achieved rapidly by redesigning chips and their holders for greater optical access. High-magnification ($>40\times$), high-numerical aperture (>1.1) microscope objectives have bulky housings (~ 2 cm) and short working distances (~ 220 μm) that are not compatible with modern LOC platform construction. Standardizing LOC construction to incorporate (1) a transparent base that is less than or equal to 170 μm thick; (2) a clearance hole in the chip holder large and/or shallow enough to permit several millimetres of translation and (3) a correspondingly greater distance between inlet and outlet fluidic ports would go a long way to enabling the use of such objectives.

Although straightforward, these improvements are not without costs. Thin substrates are more expensive and fragile

during the fabrication process. Long distances between fluidic ports require long channel lengths, which in turn necessitate higher pressures or voltages to achieve a given flow rate, and more stringent filtration procedures to avoid fouling. Finally, large open areas in the chip holder imply increased susceptibility to mechanical damage as well as larger, more expensive chips.

Nevertheless, the impact of improved optics is likely to justify these costs. For example, a common limitation is that analytes of interest are not the only source of fluorescence in LOC devices. Device materials and fluorophore adsorption to channel walls can generate enough background fluorescence to undermine the benefits of improved sensitivity. High-numerical aperture (high-NA) optics, however, would enable alternative excitation techniques that effectively eliminate background fluorescence and enable maximum sensitivity, albeit only in a limited region. One such technique is two-photon excitation, wherein the sample is illuminated with light of twice the wavelength that best excites the fluorophore, so that excitation only occurs when the fluorophore absorbs two photons simultaneously.²⁶ Since the cross-section for two-photon absorption depends on the square of the illumination intensity, this technique effectively limits excitation to the focal volume of the objective lens. Another such technique is excitation by total internal reflection fluorescence (TIRF). Here the sample is illuminated at a shallow angle (determined by the refractive index mismatch between the substrate and the sample solution) so as to create an evanescent wave. The evanescent wave decays exponentially within the sample and so limits excitation to a region within a few hundred nanometres of the channel surface. TIRF is therefore especially well suited for quality fluorescent imaging in nanofluidic systems, where channel heights are typically less than 500 nm.²⁷

Further rapid improvement of fluorescence-based LOC devices would come from development and application of new materials. For example, PDMS is the material of choice for molded LOC devices that are easy to seal and incorporate multiple layers of fluidic channels. But PDMS is itself fluorescent, with a spectrum that overlaps common

fluorophores such as FITC, Cy3 and GFP. The problem can be mitigated if analytes are labeled with fluorophores excitable at longer-wavelengths, but these are limited in number and more difficult to distinguish spectrally. Glass and quartz are viable alternatives, but require more difficult and costly fabrication methods. Non-fluorescent polymers amenable to molding and layering would be most desirable.

Fluorescent background signal may also come from fluorophores adsorbing to channel surfaces. Coatings, such as octadecyltrichlorosilane (OTS) and polyvinyl-pyrrolidone (PVP), can inhibit surface adsorption. However, more materials and protocols need to be developed and applied in order for this to become a repeatable, reliable solution.

Finally, methods for minimizing photobleaching of fluorophores could also greatly improve the quality of fluorescence measurements on-chip in the near-term. Enzymatic oxygen-scavenging systems are commonly used in sealed, finite volume samples.²⁸ However, these systems are rarely suited for LOC platforms because of the fouling that can be caused by their enzymatic protein component. Therefore, thought should be given to adapting off-chip methods for removing oxygen (*e.g.*, degassing the fluid, replacing dissolved oxygen by saturating with nitrogen) for use on-chip with limited volumes of working fluid. For example, passing the fluid over or through a chilled, hydrophobic region would nucleate bubbles of dissolved gasses that might be transported off-chip, or at least out of the main channel, by clever fluidic design.

Far-term

LOC fluorescence detection and imaging currently requires multiple bulky, off-chip components. In the long run, fabricating light sources, detectors and/or optics directly on-chip may be the best way to achieve high-sensitivity in an inexpensive and truly portable fluorescence-based LOC system. Achieving high spatial resolution on chip, however, requires detectors with smaller pixel sizes than are currently practical or the development of rapid on-chip scanning methods to avoid the optical path-lengths that magnification requires.

Several recent advances point in this direction. A portable system integrating

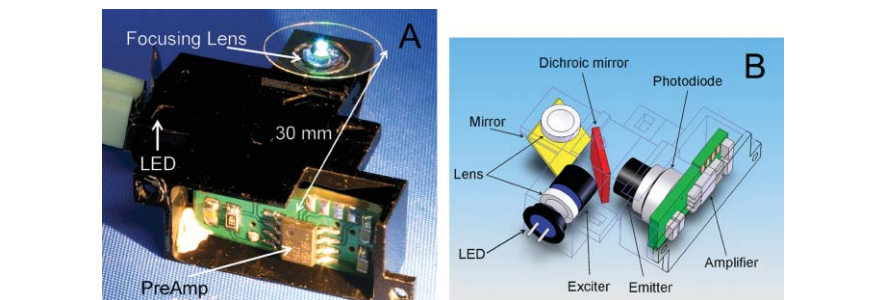


Fig. 3 (A) A photograph of the integrated detection system, assembled in a metal housing. The location of the LED light source, focusing lens, and preamplifier are indicated by arrows. (B) Schematic of the optical system. Light emitted from the blue LED is collimated by a lens, passes through the excitation filter, and is reflected from the dichroic mirror; after being redirected by a conventional mirror, it is focused on a sample by a second collimating lens. The fluorescence light from the sample is then collimated by the same lens, redirected by the mirror, passes through the dichroic mirror, and is detected by a photodiode after being filtered by an emitter filter. (Reproduced by permission of RSC publishing.)

an LED and photodiode with a LOC device was demonstrated by Novak *et al.* less than two years ago (Fig. 3).²⁹ Their system is sensitive enough for quantitative PCR, but several orders of magnitude shy of what is needed for chip-based capillary electrophoresis. Even more recently, Schonburn *et al.*³⁰ have demonstrated a Fresnel Zone plate that could be microfabricated within a LOC device, potentially providing high-NA collection without the need for bulky objectives.

Finally, the greatest possible advances may come from unconventional optical approaches that are uniquely amenable to on-chip fabrication. Imagine, for example, a chip whose bottom surface is a CCD array, instead of a thin sheet of glass, or a chip with an embedded laser source that illuminates the channel obliquely from below the fluidic ports (*i.e.* on-chip TIRF). Such devices, together with clever overlay patterns and frequency modulated detection systems, could push the performance of LOC fluorescence beyond what is currently possible with conventional optics.

Conclusions

We have discussed the need for and possible paths towards LOC high-resolution and high-sensitivity fluorescence devices. Although fluorescence is already widely used in LOC devices, it is rarely leveraged to full capacity. Development of high-resolution imaging and/or high-sensitivity detection will not only allow for orders of magnitude improvement in current LOC measurements, but also allow for novel measurements that may not be achieved otherwise. Immediate solu-

tions to benefit the research community include conventional improvements that can better integrate LOC devices with high NA optics. Farther-term solutions, such as novel designs that replace conventional light sources, detectors or optical elements with on-chip components will transform research and industry alike, lowering the barrier for entry to single-molecule studies and making fluorescence-based assays more cost-effective and portable. We hope that this focus article encourages the creation of higher-quality fluorescence LOC devices.

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