研 究 速 報

# Lab-on-a-chip: Towards the Miniaturization and Integration of Fluorescence Spectroscopy Based Detection Method onto Portable Device

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## 1. INTRODUCTION

The spectroscopy based on fluorescence phenomenon has been commonly used for many years in chemical and biochemical systems due to its numerous advantages like real-time and non-intrusive measurements, good reliability and sensitivity. It was also possible to extend this method to DNA detection by the use of fluorescent dyes that are not modifying basic properties of the target molecules. In parallel, the dye's specific attachment to DNA guaranties a good selectivity of the method.

Nowadays, the experimental set-up to perform fluorescence spectroscopy (for example in the case of electrophoresis) needs huge optical components like optical lenses, lamp and filters. But recently many applications (for example in health care or on-site measurement) require a small device able to achieve a basic fluorescence detection. So a lot of international articles are now dealing with this new concept of "lab-on-a-chip"[1], which corresponds to the integration of all the components directly on a chip to perform a complete biochemical analysis, including a detection step[2].

As the first step towards the real "lab-on-a-chip", portable devices using optical fibers have been developed so far using various materials and technologies. The main drawback that appears is the energy losses of the excitation light due to numerous connecting parts, such as the weak coupling between the external light source and the fiber, or the fiber and microfluidic channels[3]. The intensity of the fluorescent response is directly related to the excitation power so that these losses lead to a real decrease of the on-chip detection method sensitivity compared to the bulk equipments that are commercially available. Thus many efforts should be done to optimize the efficiency of fluorescence excitation in order to achieve lower detection limits.

Using PDMS (Poly (dimethylsiloxane)) technology, a very cheap and disposable device is proposed with channels dedicated to the liquid flowing, and other ones for the insertion of fibers. These channels are very close to each other and so ensure a good efficiency of the excitation of fluorescent dyes. The inserted fibers are then self-aligned with an excellent accuracy. In parallel, taking advantage of the difference of the refractive indices in air and PDMS, we have also introduced in our design an optical lens to focus more efficiently the beam on the liquid containing active species.

In the second part of this report, a new approach is detailed based on the combination of Organic Material and PDMS technologies to realize fluorescence excitation on the chip without any external light source. The Organic Materials (OM), and specifically Organic Light Emitting Diodes (OLED) seem then to fulfill all the requirements for an integrated light source. The OLED is then deposited on one side of a glass substrate, and the PDMS layer with channels network placed on its other side. An optical fiber can then collect the light coming out from the microfluidic channel and transmit it to an analyzing set-up.

## 2. A PORTABLE DEVICE WITH EXTERNAL LIGHT SOURCE

The design[4], including a PDMS layer bonded on a glass substrate, is shown in Fig. 1a and 1b. First, this design allows one to put the fiber very close to the microfluidic channel (distance from 50 microns to several hundreds of microns) without any bulge effect. The light is then guided inside the optical fiber with an excellent accuracy (losses around 10 dB/km) and launch out from the fiber near the channel. To improve the excitation efficiency, PDMS 2-D optical lenses were also implemented.

The principle of the PDMS lenses can be described as follows. The optical fiber is inserted into the channel ending by a curved interface. The two media on each side of this interface (air and PDMS) possess different refractive indices so that the light beam going through this interface will be deflected, and focused according to the curvature radius and the incident angle of the light beam, along a 2D plane parallel to the Glass wafer. The light ener-

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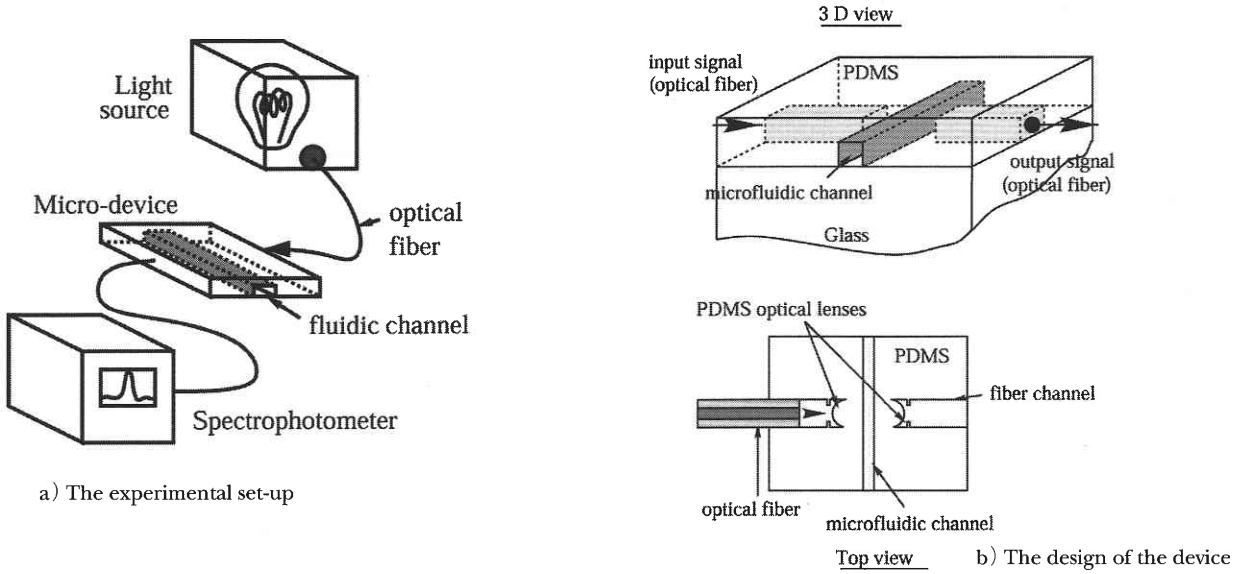



a) The experimental set-up　　b) The design of the device

Fig. 1 The portable device using optical fibers

gy density should then be increased consequently and lead to a more local and efficient excitation of fluorescent dye inside the microchannel.

### 2.1. Fabrication process

The PDMS material is a silicone rubber, which exhibits interesting properties for biological applications, such as biocompatibility, transparency in the visible range, etc. The other advantages of such devices are the low cost and the high reliability. The main technological steps to fabricate a PDMS layer are now quite well established, and can be easily found in the literature[5]. A particular attention was given to this process in order to achieve flat wall surface.

### 2.2. Experimental results

In our experimental set-up, several dyes were used as a fluorescent label to characterize the light beam inside the channels. Therefore, the set of filters (excitation light and fluorescent light) was chosen specifically in order to select the proper wavelengths. A multimode fiber with a numerical aperture of 0.22, a core diameter of 105 $\mu$m, and a clad diameter of 125 $\mu$m was used, allowing an effective and easy collection of light from the light source (Fig. 1a). The losses at 500 nm wavelength were estimated around 10 dB/km, that corresponds to the fact that more than 99.4% of the light power is transmitted per meter.

#### 2.2.2. Fluorescent emission improvement

Since one of the goals of the study is also to improve the fluorescent response from the dye by optimizing the excitation process, the effect of the PDMS lens on the light emitted by the fluorescent dye was also checked.

Then, a CCD camera with normal gain was used in order to avoid the saturation phenomenon. For these experiments, the gain

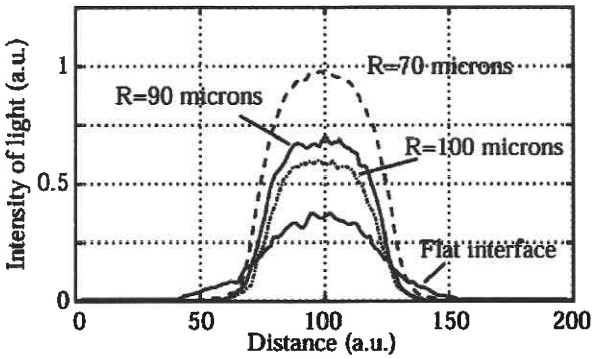



Fig. 2 Effect of the PDMS lens on the fluorescent light emission from the dye

of the CCD camera was kept constant in order to enable a comparison of the arbitrary units systems. The light intensity profiles were drawn along a perpendicular axis to the symmetry axis of the light beam, 50 $\mu$m away from the border of the channel where the dye was flowing. The results of the fluorescent light intensity using several PDMS lenses, which curvature radius R varies from 70 $\mu$m to a flat interface are reported in Fig. 2.

It shows that with the smallest curvature radius (70 $\mu$m), without changing the light source or the dye concentration, we can improve by a factor of 3 the excitation of fluorescent tags, and therefore its light emission.

#### 2.2.3. Detection limit

The detection limit is one of the most important parameter in order to estimate the efficiency of a detection device. In order to check the influence of lenses on this characteristic, systematic studies were done with two types of interfaces (70 $\mu$m curvature radius and flat interface) and two different distances between the fiber and

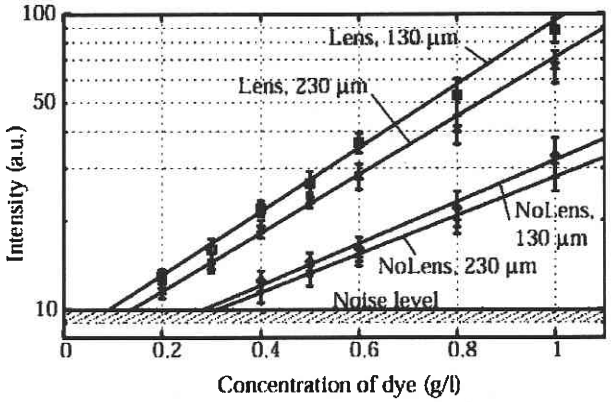
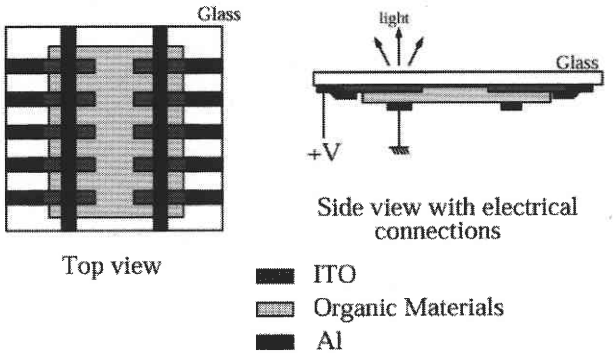
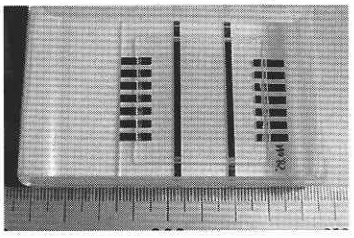



Fig. 3 Response of the labeled solution flowing inside a microchannel to an excitation by an optical fiber: comparison of the design with lens (70 $\mu$m curvature radius lens) and without any lens (flat air-PDMS interface). The distance between the end of the fiber and the microfluidic channel are indicated in the graph.




a) The design of the OLED



b) The OLED fabricated on glass substrate, with the encapsulation system

Fig. 4 OLED as an integrated light source a) design of the Organic Light Emitting Diode on glass substrate, including ITO, Organic Materials and Al deposition processes. The electrical contacts are also shown on the right figure in order to obtain light emission through the substrate (the encapsulation is not shown in this drawing). b) a photo of the corresponding device

the microfluidic channel (130 $\mu$m and 230 $\mu$m). For these experiments, the microfluidic channel was 50 $\mu$m in width and 130 $\mu$m in height and the gain of the CCD camera was set at 0 dB. The results are presented in the Fig. 3.

The small curvature PDMS lenses (typically around 70 $\mu$m radius) decrease the width of the beam close to the lens. This focusing phenomenon (the spot width is 30% smaller than the flat interface one) results in improvement of the excitation and emission around three times brighter than the set-up with a flat interface using the same dye concentration. Thus Fig. 3 shows the four linear curves we obtained depending on the interface shape (with or without 70 $\mu$m curvature radius lens) and the distance between the fiber and the microfluidic channel (130 $\mu$m and 230 $\mu$m).

The upper set of curves corresponds to the design including a PDMS 2-D optical lens. Compared to the lower curves (flat interface), the slope is bigger and also the solution with 0.2 g/l dye was clearly detectable. That leads to a detection limit around 0.1 g/l with this solution. Unfortunately, it was impossible to detect this concentration with a good accuracy. The detection limit is estimated around 0.3 g/l in the case of a flat interface, three times higher than the set-up with small curvature lenses.

If the two upper curves are compared to each other (or the two lower one), the distance between the fiber end and the microfluidic channel seemed to contribute also to the excitation efficiency. By setting the microfluidic channel 100 $\mu$m closer to the fiber, we could slightly improve the detection limit for both configurations.

The use of lenses increased the slope of the linear dependence of the light intensity upon the concentration of fluorescent labels. It should be advantageous for quantitative measurements because the larger slope means increased sensitivity to the concentration.

The present 2D optical lens is useless if we consider the Z-direction, perpendicular to substrate surface. This phenomenon, coupled with the roughness of the vertical side wall that we cannot control with a better accuracy than a few tenth of the excitation wavelength going through the lens, could explain the difficulty in focusing the light beam inside a smaller diameter sphere. The aberration also was not taken into account in that study. These parameters should be carefully considered in order to improve the presented results.

## 3. ORGANIC LIGHT EMITTING DIODE AS AN INTEGRATED LIGHT SOURCE

As a step forward the complete integration of all the elements required to perform a detection on a portable chip, the on-chip light source is a very challenging research. Two approaches were recently proposed and developed, based on Vertical Cavity Surface Emitting Laser (VCSEL) and Organic Light Emitting Diode (OLED). In collaboration with the Arakawa laboratory, we then chose the OLED technique to fabricate the on-chip light source.

Following our design as shown in Fig. 4, the organic materials and the two electrodes (the lower one ITO, and the upper one Aluminum) are deposited on one side of a glass substrate. The PDMS layer including the microchannel in which the liquid is flowing is bonded on another glass. Then the two free glass sides are juxtaposed without any bonding. This technique enables one to use the same OLED with several PDMS layers, and PDMS and OLED are fabricated separately. Thus we didn't have any problem of technological incompatibility between the two processes.

The first concentration measurements were carried out with optical fibers and PDMS layer, pointing out some trouble due to the broadband spectrum of the light emitted by the OLED.

### 3.1. The Organic Light Emitting Diode: deposition process and characterization

The process to fabricate the light emitting diode on a flat glass substrate has been previously reported in details[6]. To summarize briefly, it consists of two Organic Materials ($\alpha$-NPD and Alq$^{\dagger}$ with some doping material) deposited between two electrodes (IndiumThinOxide and Aluminium). When a current is flowing between the two electrodes through the organic materials, light is emitted through the ITO and Glass plate around 510 nm (Green color) as shown in Fig. 5.

Then a PDMS layer was stuck on the other Glass plate side and a solution containing fluorescent dye was introduced. The fluorescent label was chosen in order to match the OLED output light and the dye absorption spectra.

### 3.2. Concentration measurements

Several solutions were introduced in the microfluidic channel with fluorescent dye concentration ranging from 100 $\mu$M to 0 $\mu$M (deionized water). The fluorescent light emitted by the illuminated dye was collected by an optical fiber and then analyzed by a PMT after passing thought a polymer filter (design of the PDMS layer very close to the one depicted in Fig. 1b). The results are shown in Fig. 6.

The voltage measured corresponds to the difference of the output signal delivered by the PMT when the microfluidic is filled with air and liquid containing the fluorescent dye. When the channel is empty (no liquid), a large amount of light emitted by the OLED is reflected by the top wall due to air-PDMS refractive index mismatch, and launched into the fiber. Although a polymer filter is used, the OLED output spectrum is non-negligible at the wavelength we should detect the fluorescent emission, and leads to a high background signal.

Thus, by filling the channel with liquid, two phenomena appeared at the same time: the refractive index mismatch along the microfluidic channel wall decreases so that the PMT output signal decreases. If the solution contains some fluorescent dye, the fluorescent light collected by the fiber will increase the output

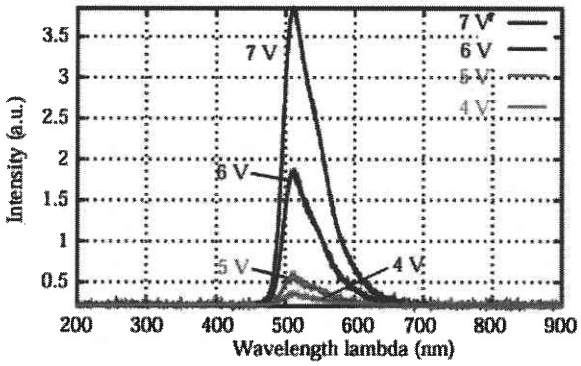



Fig. 5 Optical characterization of the OLED fabricated on a glass substrate: spectrum of the light emitted by this organic diode depending on the input voltage

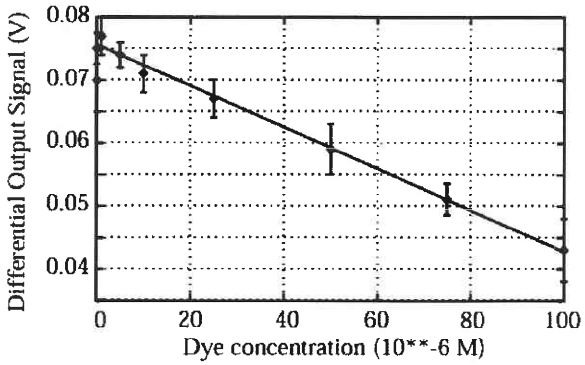



Fig. 6 Output signal of PMT versus the concentration of fluorescent dye

voltage of PMT. Then, the differential output signal decreases when the dye concentration is getting higher.

In Fig. 6, a linear dependence was obtained, but the measurements exhibit large error bars that make difficult to use this device at low concentration (below 5 $\mu$M). These error bars should disappear if we use another experimental set-up where the microfluidic channel is permanently filled with liquid and fluorescent active chemicals or species are flowing within localized area (band shape), which corresponds to, for example, separated DNA inside microchannel by capillary electrophoresis.

## 4. CONCLUSIONS AND PERSPECTIVES

We have presented in this report a new approach with optical fibers directly inserted into channels that end with PDMS 2D optical lenses. This method exhibit several advantages, such as a cheap microdevice with self-aligned fibers put close to the microfluidic channel. The efficiency of these lenses in terms of the fluorescent dye excitation were characterized. The results demonstrate non-negligible improvements compared to the commonly used flat interface. The 70 $\mu$m curvature radius PDMS 2-D optical lens exhibits optimized properties. The dye excitation was enhanced by

a factor 3. In terms of sensitivity, these lenses yield a lower detection limit threshold by a factor of 3.

The present technique doesn't require any specific fabrication step and can be easily applied to portable devices based on a PDMS layer and optical fibers. By a slight modification of the photolithography masks, the performance of such devices can be improved in terms of excitation efficiency for both absorption (not discussed in this report) and fluorescent measurements.

This design can also be applied to other detection method as UV absorbance, because the PDMS material absorption is small from the near UV to the IR wavelength. Depending on the application, to set the right fiber optimized for a certain wavelength is sufficient to adapt the present method to other domain of application.

In the second part, a new method based on Organic Materials was presented in order to integrate the light source directly on the chip. By combining the OLED technology with the PDMS one, a microdevice with a detection system based on optical fiber and PMT was proposed and fabricated. The first results with fluorescent dye were also reported and exhibit a low sensitivity compared to bulky equipments such as fluorescent microscope. Concerning the detection of capillary electrophoresis bands, this set-up should fulfil the requirements and will be tested in the near future.

But these results pointed out some important drawbacks (such as OLED broadband emission, roughness of channel's walls, etc). In order to improve the sensitivity of the method, we should solve the problem of direct coupling between light source and fibers. And another problem is the use of polymer filters. These films are very thin (less than 1 mm thick) and smooth, but their transmission coefficients are bad compared to the glass filters. Both approaches should be tested and should lead to better results in terms of sensitivity.

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† a-NPD: N,N'-di (alpha-naphthyl) -N,N'-diphenyl-1,1'-biphenyl-4,4'-diamine Alq : tris (8-quinolinolato) aluminum

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