Characterization of a Laser Induced Fluorescence Detection System for Microdroplets Fluorescence Quantification

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Abstract: On this work a laser induced fluorescence system for microdroplets fluorescence quantification is described in detail, and it is characterized considering flow rate, laser power and fluorophore concentration. At the end, results of a microdroplet essay are presented.

1. Introduction
On recent years the technology known as Lab On a Chip have reached an important success on micro instrumentation for chemical and biochemical research [1,2]. This technology uses microfluidic devices to transport and manipulate small quantities of fluids and biological specimens through micro channels where reactions, separations and combinations take place [1]. Among the vast possibilities that this technology offers, there exist the option to generate micro droplets between a two phased fluid inside micro channels, and used those droplets as micro reactors that allow individual and isolated reactions inside each of them [2]. These can be fused, sorted, cooled and heated, providing an adequate environment to perform both chemical and biological essays [2].

Nowadays, in the microfluidic technology world, two main detection methods have been implemented: electrical and optical [3]. Typically, electrical detection methods are based on impedance measurement and optical methods are often based on fluorescence. Both of them have the advantage of being non-invasive techniques; however, optical methods usually present higher sensitivity, selectivity and resolution [3,4]. The optical method based on laser-induced fluorescence (LIF) has been well established on capillary electrophoresis [5]. A very important advantage of LIF is the ability to decrease the sample size down to single molecule [4], which in combination with the use of micro droplets, result in a high throughput screening for enzymatic assays, protein expressions, protein crystallization, just to mention a few applications [5].

Various LIF systems have been developed [3-5] and their characterization is mainly focused on the improvement of the detection limits [5,6], where fluorescein concentrations of pM orders have been reported [6]. However, there are other factors that affect analytical sensitivity such as laser power, sample size and volume, photo bleaching and flow velocity [7]. For instance, in focal LIF systems the relative laser power becomes very high when the focused laser beam is very fine. This increment of energy occurs because the area where the energy is applied is reduced from millimeters, of a common laser beam, to about tens of micrometers, generating a high energy concentration that is reflected on relative power of several orders of magnitude higher than the original source[8]. This high power makes photo bleaching phenomena an important factor on the intensity of fluorescence emitted. For this reason, the fluorescence intensity not only depends on fluorophore concentration, but also on laser power and flow velocity [7].

In this work we present a LIF system implementation for micro drops and its characterization based on the relevant system parameters discussed above: fluorophore concentration, flow velocity and laser power.

2. Experimental methods

2.1 Fabrication of microfluidic chip
The microfluidic chips were fabricated using standard PDMS soft lithography technique based on mold/replica. Molds were made using photolithography of SU-8 over a silicon wafer. The microchannels were characterized using a white light optical interferometer (Polytec MSA-400) and the dimensions were 102 \(\mu\m\) high and 98 \(\mu\m\) wide, as can be observed in figure 1.b.

2.2 Micro-droplet formation principle and reagents employed
The microdroplets are generated by intersecting flows of mineral oil and a fluorescein solution inside the micro channel circuit, forming an emulsion. This was stabilized adding span 80 to mineral oil (2 %). Fluorescein concentrations were: \(1\times10^6\) (\(M; \text{pH} 7\)) for continues flow experiments and \(1\times10^5\) (\(M; \text{pH} 7\)) for micro droplets essays.
2.3 Apparatus and equipment
A schematic diagram of the LIF detection system characterized is shown in figure 1.a). An optically pumped semiconductor laser 488 (sapphire 488, Coherent) was used as light source to stimulate the fluorescence. The optical trajectory is described as follow: the laser beam was reflected on a 488 nm dichroic mirror (Di01-R488 Semrock) through a fluorescence port of an inverted fluorescence microscope (DM IL LED, Leica) an reflected on a second dichroic mirror (FF660-Di02, Semrock) placed on the microscope filter holder. The laser line was focused by the objective (20x, NA=0.30, Leica) and pointed to the micro channel. The fluorescence light emitted by the sample (the micro-droplet) was collected by the objective, later reflected by the dichroic mirror (FF660-Di02) and passed through the first dichroic mirror (Di01-R488) and through a band pass filter 500-550 nm (FP02-525/50-25, Semrock). Finally the light was concentrated by a plano-convex lens (LA1951, Thorlabs) before being detected by an avalanche photodiode (APD-100, Hinds Instruments). The signal from the APD was recorded by a data acquisition card (USB-6351, National Instruments) and processed using both LabVIEW signal express and Matlab.

![A LIF system implemented over an inverted fluorescence microscope](image)

**Figure 1.** a) A LIF system implemented over an inverted fluorescence microscope; b) Profile of a micro-channel characterized using Polytec MSA-400 non invasive topography system; c) Microfluidic PDMS chip employed for microdroplet fluorescence essays.

2.4 System characterization methodology
Continuous flow experiments were carried out on a microfluidic chip with a single straight micro channel on it. The syringe pump was loaded with the fluorescein solution previously described and a constant flow rate was set. Then, the laser was focused on the micro channel and a fixed power was selected. We started the characterization with a continuous flow of 50x10^-6 L/h and laser power of 4 mW, once the flow and laser power were stable the fluorescence was quantified. Then we set the laser power on 6 mW, after its stabilization a second lecture was obtained and so on up to 10 mW. The same procedure was applied to different flow rates and an additional test was made fixing the laser power on 4 mW and using three additional concentrations (5x10^6, 10x10^6 and 50x10^6 M; pH 7). Finally, a microdroplet fluorescence essay was performed using a microfluidic chip shown in figure 1.c, using a fluorescein solution (10x10^6 M; pH7) and a flow rate of 100x10^6 L/h of both fluorescein solution and mineral oil.

3. Results and discussion
The results in figure 2 shown the sensitivity of the LIF system implemented to relative small changes in the flow rates and laser power. A previous report [7] detected a comparable behavior for flow rates from 0.02x10^-6 L/m to 1x10^-6 L/m. In this study we confirm this tendency and proved flows from 50x10^-6 L/h to 200x10^-6 L/h. These higher rates are employed to form microdroplets with higher frequency rate and same tendency was observed in all the range studied.
Note that the laser power also has a high impact on fluorescence intensity for a flow rate of $200 \times 10^6$ L/h and at laser power of 10 mW the signal was about 60% higher than the signal observed at the same flow rate and 4 mW.

Figure 3 (left) shows the fluorescence intensity versus fluorescein concentration plot. Note that for four different concentrations the signal intensity increases with the flow rate. The explanation behind this is that higher flow rates correspond to less photo-bleaching effects, due to the fact that the same portion of fluorescein is pointed by the laser during a smaller amount of time, obtaining higher amplitude of the read signals. The linear trend is consistent. Finally, figure 3 (right) shows the signal acquired when a microdroplets essay is performed with flow rates of $100 \times 10^6$ L/h for both oil and fluorescein, achieving 16 Hz microdroplets formation.

4. Conclusions
On this work a LIF system was implemented and the effects on fluorescence of varying fluorophore concentration, flow velocity and laser power were determined. The expected tendencies on photobleaching phenomena and laser power effects were confirmed. Further work is required to find the cross relation between laser power and flow rates.

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6. References