



Microfluidic lab-on-chip sensor capable of performing improvement optical properties

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ABSTRACT

In this study, a microfluidic lab-on-chip sensor system that may enhance optical properties-based (fluorescence intensity) detection of liquid sample concentration is described. The microfluidic lab-on-chips sensors are Y shapes made of PMMA substrates by laser CO₂ manufacturing techniques at power 60 watt & travel speed of laser (250, 350 & 500) mm/s. The design of the sensing system includes optical fibers, reading from laser-induced fluorescence. It is suitable for low-cost, straightforward, & user-friendly sensing applications. The findings show that the addition of carbon nanotubes to a lower concentration made it feasible to assess improvements, as well as the concentration-related to fluorescence. Measurements were made using rhodamine B solutions in the range of 100-1.5625 mg/L, & carbon nano tubes (6.625, 3.125, 1.5625 mg/l).

Keywords: Lab-on-chip, microfluidic, laser-induced fluorescence, surface roughness, rhodamine B.

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1. Introduction

Nowadays, microfluidics has a wide range of uses in the sciences of chemistry, biology, & engineering. One major obstacle to the development of these devices' industrial & medicinal uses is their optimal manufacture. Fortunately, a number of novel strategies have been developed recently that could be useful in both academic & commercial domains [1]. In addition to delivering additional benefits including high quality factor, reagent minimization, real-time measuring capabilities. Due to short diffusion times, lower process costs, non-contact measuring capabilities, etc., these contemporary technology gadgets provide customers high sensitivity & dependability. Due to the integration of microfluidic devices with electromechanical systems for point-of-care testing, sensing may now be performed without a complex laboratory setup or specialist personnel. In addition, higher sensitivity & sensing capabilities may be achieved because to the microscale's large surface-to-volume ratio (relative to the macroscale). Due to these advantages, merging microfluidics with other sensing technologies paves the way for future biosensing applications. [2]. Because of this, interest in the viability of using PMMA substrates for microfluidic

applications has grown recently. PMMA has a number of significant advantages over quartz & glass, including resistance to hydrolysis & chemical inertness in neutral aqueous solutions. Another advantage is that PMMA is less expensive. In addition, PMMA is a solid that does not have pores, which lessens the effect of pollution brought on by the bimolecular adsorption that occurs at the walls of microchannels. There are a few different approaches that have been proposed in the research literature for the production of PMMA substrates that are used in microfluidic applications. These approaches include hot embossing, wire imprinting, & excimer laser ablation. In recent years, a number of different groups have investigated the possibility of patterning PMMA substrates using technologies that are based on lasers. Microchannels were scribed onto polymeric substrates using a CO₂ laser system that was primarily designed for the marking of goods in an industrial context [3]. This was one of the first investigations to be conducted on the topic. Because of its high level of absorption in the middle infrared spectrum, polymethyl methacrylate (PMMA) lends itself very well to processing by CO₂-based lasers [4]. When it comes to IR laser systems, a computer-controlled CO₂ laser

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setup is used for the ablation of the designed pattern on the surface of the desired substrate while being subjected to continuous IR radiation. On the other hand, when it comes to UV lasers, a high energy light is used for the targeted breakdown of the material in very short pulses. In all approaches, the channel dimensions are determined by the amount of power that the laser outputs, the pace at which it scans, & the distance that separates the surface of the substrate & the laser focus plane. In spite of the fact that both approaches have been employed for the purpose of microfabrication, ultraviolet lasers are not often used due to the low level of repeatability they provide. [2]. Polymer substances like poly-methyl acrylate (PMMA). Since then, there have been a few publications on the use of a CO2 laser to fabricate disposable integrated devices for genetic testing in polycarbonate for a variety of biomedical applications [5]. CO2 laser micromachining offers great adaptability & cost-effective production among the many fabrication techniques. Without the need for molds or specialist equipment, commercially accessible tabletop laser cutters may be utilized to create a wide range of microfluidic designs with various specifications, allowing for quick prototyping & iterative design of microfluidic devices [6]. One of the most accurate methods for monitoring temperature at the macroscale is laser-induced fluorescence (LIF). With this technique, a temperature-sensitive fluorescent dye is dissolved in the fluid of interest & lit to light [7]. Confocal LIF systems' broad use in microfluidic chips seems to have been hampered by both the optical system's difficult construction & miniaturization requirements as well as its complex & essential structure. In addition to the confocal arrangement, other nonconfocal LIF detection techniques based on orthogonal or bevel incident laser arrangements were also developed for connection to chip-based systems, which have the advantages of a relatively simple construction & straightforward system assembly. In the earlier LIF approach, the laser beam was normally focused on the microchannel with a bevel incidence angle, such as 45°, & the emitted fluorescence was detected in the direction perpendicular to the microchip. [8]. LIF is a non-invasive method for detecting changes in the spatial distributions of scalar variables, such as temperature fields & tracer dye concentration fields [9]. Among all of these detection techniques, fluorescence detection is a sample-free detection technique that significantly eliminates sample interference. Laser-induced fluorescence (LIF) detection is a unique fluorescence detection technique that has a very high detection sensitivity. LIF is the best detecting technique because of its compact size, cheap cost, & simplicity of operation [10]. In this work, PMMA was used as the bulk material for CO2 laser ablation manufacturing of multilayer microfluidic sensor devices. On PMMA substrates, microchannels were created using CO2 laser ablation device, & many layers were subsequently attached concurrently using chloroform bonding techniques. This research also examined the depth, chip width, & surface roughness of the manufactured microchannel. Several PMMA-based microfluidic devices were fabricated & tested as a demonstration of the proposed fabrication techniques. The

application of PMMA material in polymer microfluidics could provide a new alternative & have broad potential applications in biological, chemical, medical, & environmental fields for some point-of-care applications.

2. Materials & Methods

2.1 Materials & Reagents

This paper examined PMMA extruded sheets with a 2.5 mm thickness that were purchased from Shenzhen Xintao Co Ltd., Shenzhen, PRC as the substrate & cover for microfluidic chips. Chloroform (Minimum Test, GC, 99.0 percent, Chemical Zone, Umber Nath 4210501. India) used as an adhesive between layers for PMMA microfluidic chips. The PMMA microfluidic devices were tested using Rhodamine B for optical clarity. (Product Description Product Name: Basic Violet 10, C.I. No.: 45170, CAS No.: 81-88-9 Molecular Formula: C₂₈H₃₁CIN₂O₃, Molecular Weight: 479.01, (Q/HG 33259-97) from Tianjin Yongda Chemical Reagent CO., Ltd., Tianjin, China).

2.2 Experimental Set Up

The commercial CO2 laser equipment from which the PMMA substrate was engraved uses air cooling (LiaochengJK-4060, Laser Engraving Cutting, Machine, China). The laser operates in continuous wave mode at a frequency of 5 kHz, with a wavelength of 10.6 μm & a maximum output power of 60 watt. The dispersion of beam intensity was almost gaussian. The microfluidic chips are Y-shaped, the laser power is 60 watt, and the beam travel speed may be programmed to be between (250, 350, & 500 mm/s). The CAD software's program used to estimate the laser's output power & adjusting beam travel speed was used to directly manufacture the planned microchannel designs. In order to provide fluid access to the microchannels, access holes were also created using the CO2 laser. Next, the chamber was built by joining 2 PMMA sheets together using chloroform bonding.

Microscopes (5X-180X Manufacturing Zoom Stereo Microscope with 144-LED Ring Light & 10MP Digital Camera, Am scope, United States) were used to measure the widths & depths of microchannels. The magnifying powers offered by this microscope include (5X). Iran's microfluidic chips were measured for depth & roughness using a laser confocal microscope. A schematic representation of the laser machining apparatus utilized to etch the PMMA substrates can be shown in Fig. 1. For the purpose of detecting transmitted light, the fluorescence light (830 m FC/PC, connectors, MM stainless-steel Ferrule, Thorlabs) gathered via the detection fiber is attached to temporary connections (830 m FC/PC, connectors, MM stainless-steel Ferrule). A detection fiber collects fluorescence light, which is then directed toward a compact CCD spectrometer (CCS200 - Compact Spectrometer, Extended Range: 200 - 1000 nm, Thorlabs).

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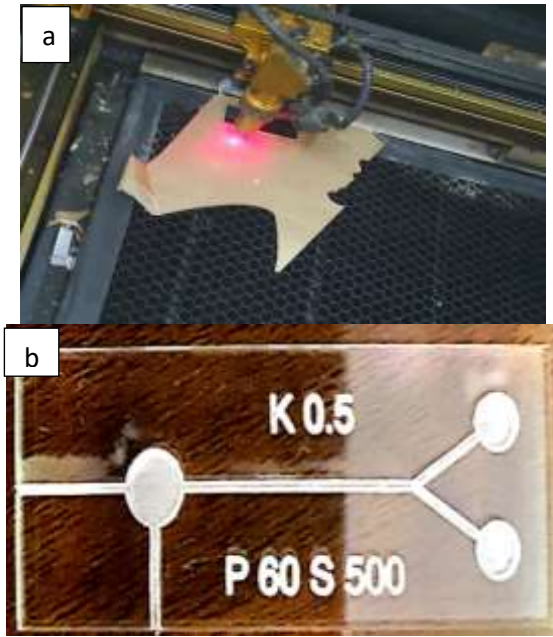


Fig. 1a) Fabrication process of the microfluidic device via laser ablation CO₂ of the microchannels & port openings b) microfluidic chip after fabrication.

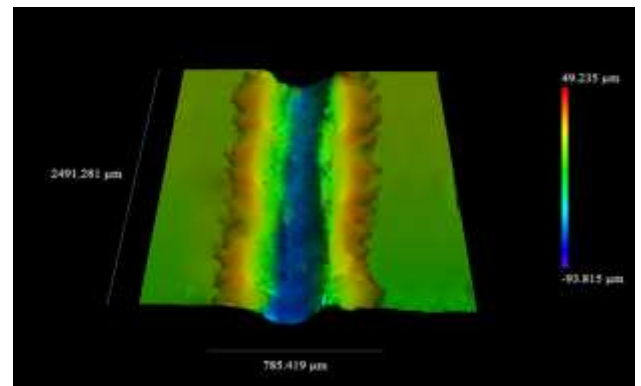
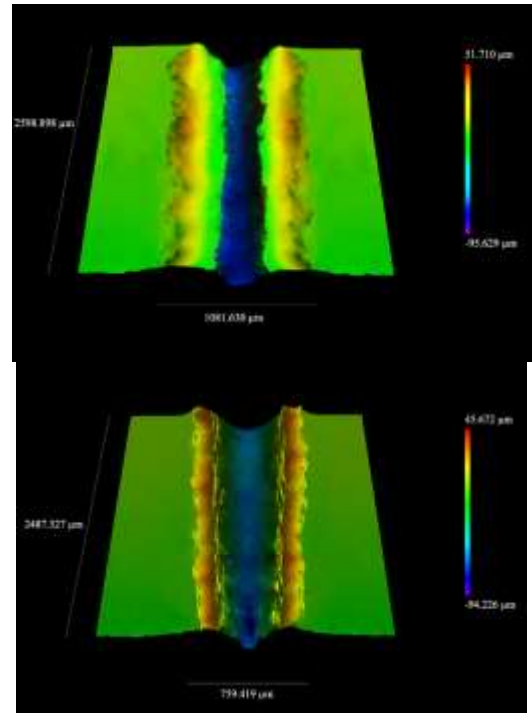


Fig.(2).Using a confocal laser scanning microscope with a 20-watt laser & a scan speed of 250, 350, or 500 mm/s, the device's depth is shown.

3. Results & Discussion
3.1 Channel Profile

The thermal diffusivity of PMMA, the laser's intensity distribution, laser power, & beam travel speed all affect the microchannel's profile. All along, a consistent spacing was maintained between the substrate & the laser beam. Since PMMA has a relatively low thermal diffusivity, the intensity distribution mostly defines the channel profile. The microchannel has a Gaussian-shaped profile as a result of the laser beam's energy being distributed Gaussianly.

A pool of molten polymer is formed on the workpiece surface where the laser beam makes contact with it as it advances over the surface. The heated gas of the vaporizing polymers drives the pool away from the hot point in all directions, while the majority of the melted material re-solidifies in the aftermath of the beam, leaving behind minor bumps on the microchannel's borders.

The bump height is modest when compared to the depth. This allows the moving laser beam to carve into the workpiece structures like channels & wells. Figure 2 shows a micrograph of the cross section of a microchannel with a Gaussian-like profile.

3.2 The Effect of Laser Power and Beam Travel Speed on Microchannel Depth

On the substrate made of PMMA, the depth of the microchannels is demonstrated to be a function of the laser power despite the fact that beam travel speeds are varied. It is not hard to see that the laser beam travel speed has a direct correlation to the increase in the depth of the microchannels. As can be seen in Table 1, increasing the laser power to 60 watts while simultaneously decreasing the beam travel speed to 250 mm/s would result in more heat being given to the PMMA surface.

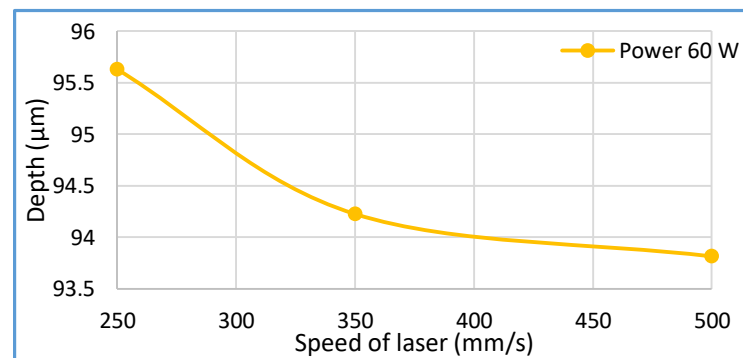
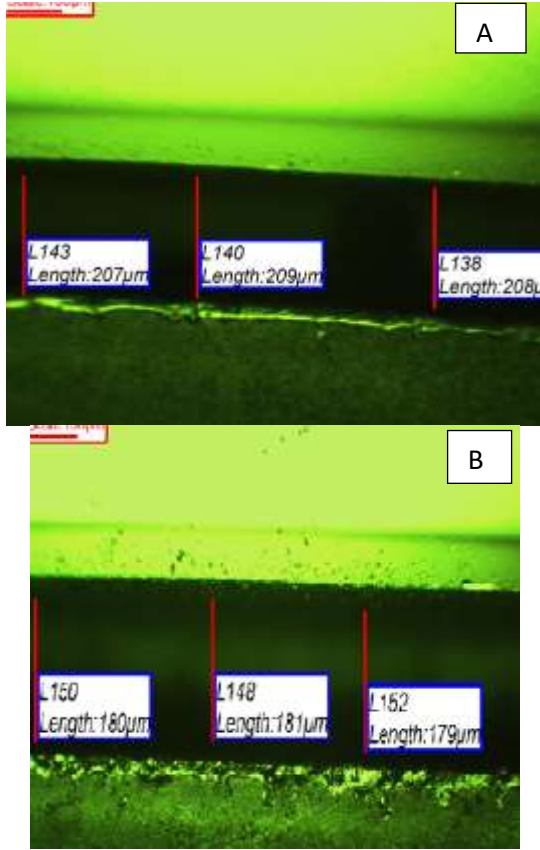


Fig (3).Microfluidic chip depth as measured via confocal laser scanning microscope at 60watt laser power & 250, 350, 500 mm/s laser travel speed.



3.3 The Influence of Laser Power and Beam Travel Speed on Microchannel Width

The widths of the microchannels are shown as a function of the laser power in figures (4,5), which demonstrate this relationship for a variety of beam travel rates. It has been shown that the breadth is influenced both by the strength of the laser and the speed at which the beam moves. Table 1 indicates that larger microchannels may be generated by increasing the laser power to 60 watts and decreasing the beam travel speed to 250 mm/s. This was possible since the PMMA surface temperature was increased.



Figure(4).Showing width of the microfluidic chips via the optical microscope images power of laser is constant at 60watt&travel speed is a) 250 mm/s b) 350 mm/s, c) 500 mm/s.

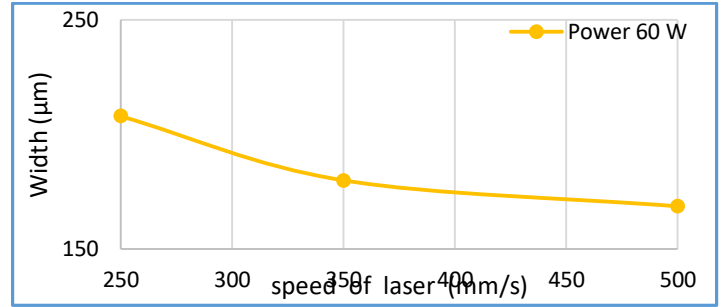


Fig.5.Measurement of PMMA microchannel characteristics at various speeds (250, 350, 500 mm/s) & constant power (60)W.

Table 1. (Dimensions of the microfluidic PMMA devices Y shape at fixed power 60watt)

Travel speed(mm/s)	250	350	500
Width (µm)	208 ± 2	180 ± 2	170.33 ± 2
Depth (µm)	95.629	94.226	93.815

3.4 Surface Roughness Effects of Laser Power & Beam Travel Speed

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The surface roughness of the laser ablated microfluidic chips was also measured with the help of the laser confocal microscope. Figure 2 shows the laser confocal microscope image of the laser-ablated microchannel with a laser power of 60wattand a travel speed of 250,350,500 mm/s. The surface roughness was measured at the inner wall of the fabricated microchannels (excludes the bulge edges alongside the microchannel). As shown in Fig. 6, generally, higher laser scan speed results in lower surface roughness, & laser power have no significant influence on the microchannel surface roughness. To improve the surface roughness, some conventional approaches include chemical treatment with the organic solution & multiple scans along the same route.

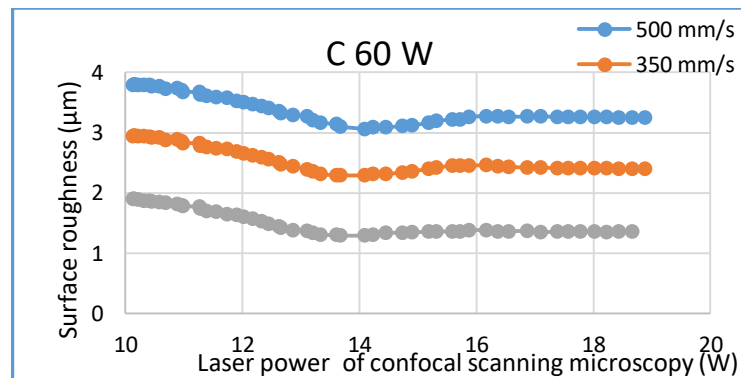


Fig. 6. Showing the Surface roughness of the microfluidic devices via Confocal Laser Scanning Microscope at power (60) W straight channels, at various travel speeds (250, 350, 500 mm/s).



3.5 Optimization of the microfluidic sensor LIF assay method

These optimizations included the effect of the addition CNTs on the rhodamine B solution performance (concentration, & additives) affect the fluorescence intensity, also the microfluidic sensor devices that of (Dimensions & surface roughness). For many applications, Rhodamine B (RhB) is utilized as the sensitive dye. Both water & organic solvents may be used to dissolve this color. Furthermore, RhB absorbs

light. at $\lambda_{abs} \approx 535 \text{ nm}$ & fluoresces throughout a spectrum of wavelengths of $525 \text{ nm} \leq \lambda_{em} \leq 580 \text{ nm}$ when dissolved in either water or ethanol [7]. Since a portion of the dye's fluorescence occurs at higher wavelengths & it can be easily excited via a Nd: YAG laser emitting in the green, these characteristics are very helpful in the microfluidic sensor because they make it easier to separate the emission wavelength from the illuminating wavelength. Tables 2, 3, & 4 provided the findings.

Table 2. Fluorescence intensity for rhodamine B in the microfluidic lab on chip sensor

Microfluidic lab-on-chip sensor	Laser power 60 W		
	250	350	500
Travel speed of laser (mm/s)			
Fluorescence of Rhodamine(100 mg/l)	0.751739	0.718461	0.609109
Fluorescence of Rhodamine(50 mg/l)	0.521419	0.489865	0.478441
Fluorescence of Rhodamine(25 mg/l)	0.406681	0.404563	0.300795
Fluorescence of Rhodamine(12.5 mg/l)	0.279633	0.267508	0.178978
Fluorescence of Rhodamine(6.25 mg/l)	0.161	0.151684	0.090552
Fluorescence of Rhodamine(3.125 mg/l)	0.055579	0.06704	0.083339

Table 3. Fluorescence intensity for CNTs in the microfluidic lab on chip sensor

Microfluidic lab-on-chip sensor	Laser power 60 W		
	250	350	500
Travel speed of laser (mm/s)			
Emission wavelength 574.659 (nm)			
Intensity of Fluorescence Carbon nanotube (6.25 mg/l)	0.048052	0.0277	0.0204
Intensity of Fluorescence Carbon nanotube (3.125 mg/l)	0.04574	0.0234	0.0207
Intensity of Fluorescence Carbon nanotube (1.5625 mg/l)	0.028589	0.0219	0.0114

Table 4. Fluorescence intensity for rhodamine B with CNTs in the microfluidic lab on chip sensor

Microfluidic lab-on-chip sensor at 60 W	Speed (250 mm/s)		Speed (350 mm/s)		Speed (500 mm/s)	
	λ (nm)	Intensity	λ (nm)	Intensity	λ (nm)	Intensity
Emission wavelength (nm) & fluorescence intensity (a.u)						
Intensity of Fluorescence Rhodamine B (3.125 mg/l)	585.0366	0.055788	584.6825	0.06704	584.447	0.083339
Fluorescence Rhodamine B (3.125 mg/l) & Carbon nanotube (6.25 mg/l)	580.1992	0.131425	575.3659	0.09305	575.837	0.078032
Fluorescence Rhodamine B (3.125 mg/l) & Carbon nanotube (3.125 mg/l)	577.723	0.11817	575.8373	0.083265	575.484	0.06437
Fluorescence Rhodamine B (3.125 mg/l) & Carbon nanotube (1.5625 mg/l)	578.0767	0.086779	576.544	0.067112	575.719	0.059797
Concentration of Rhodamine B & Carbon nanotube	λ (nm)	Intensity	λ (nm)	Intensity	λ (nm)	Intensity
Fluorescence Rhodamine B (2 mg/l) & Carbon nanotube (6.25 mg/l)	579.3737	0.027348	580.6709	0.022805	581.379	0.022727
Fluorescence Rhodamine B (2 mg/l) & Carbon nanotube (3.125 mg/l)	582.5583	0.019714	582.7943	0.01733	583.266	0.016987
Concentration of Rhodamine B & Carbon nanotube	λ (nm)	Intensity	λ (nm)	Intensity	λ (nm)	Intensity
Fluorescence Rhodamine B (1.56 mg/l) & Carbon nanotube (6.25 mg/l)	574.659	0.022626	575.4838	0.019259	576.78	0.014844

4. Conclusion

We demonstrated the low concentration of rhodamine B, which has lower fluorescence intensity & improvement fluorescence via addition carbon nanotubes (CNTs) only a proportion of 1:1 using a Lab-on-chip microfluidic sensor. Furthermore, microfluidic device had higher intensity at power 60 watt, travel speed of CO₂ laser 250 mm/s. As a result, this method, which doesn't need solvents or significant quantities of solubilizing agents, may help create

formulations with higher safety ratings than typical goods. This technique, which makes use of a microfluidic device, is shown to give both quality robustness & the useful benefit of high-throughput formulation optimization at a small scale. To create pharmaceuticals quickly & affordably, we may prepare nanoparticles using a minimal quantity of CNTs, add them to solution, & flow them in our microfluidic system. We think that these discoveries might provide a fresh formulation choice that can get over the typical development challenges for medication candidates with low fluorescence intensities.



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