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RELAZIONE ANNUALE SULL'ATTIVITA' SVOLTA

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Dottorando	VADAKKUM VADUKKAL UDITH KRISHNAN
Tutor	Dr. Antonio Ancona
Coordinatore	Prof. Giuseppe Iaselli
Corso di Dottorato	Physics
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In piena coerenza con le attività previste dal progetto nell'ambito del quale è stata finanziata la borsa di dottorato, si presenta la relazione annuale dell'attività svolta.

Data: 25-7-2019

Firma dottorando:

Dipartimento Interateneo di Fisica "*Michelangelo Merlin*"

Dottorato di Ricerca in Fisica XXXII ciclo

PhD: Udith Krishnan

2nd year activity report

Introduction

In recent years, there has been a growing interest in developing miniaturized microfluidic systems collectively called micro total analysis systems and lab on a chip system. A Lab on a Chip (LoC) can be defined as a device in which multiple laboratory techniques are integrated in a chip of few square centimetres. Lab on a chip field mainly relies on microfluidics technology. Microfluidic technology used in lab on a chip devices allows to manufacture lot of microchannels. The microchannels network design must be precisely elaborated to achieve the desired features. Among the most interesting applications of LoCs technology, are fully-integrated portable microfluidic devices, called Point-Of-Care Test (POCT), capable of performing diagnostic tests directly at the patient's site (hospital, outpatient clinic, home) delivering the result in a few minutes after taking a small amount of biological sample (blood, tissue, etc.) without the need for particular knowledge or professional skills. The ever-increasing need to miniaturize POCT microfluidic devices to reduce the costs and quantities of analytes and samples to be used (such as limited biological samples to be taken on the field) and to have a perfect integrability and portability of the analytical systems pushes for the search for new prototyping technologies. LoCs have tremendous potential for application in various fields of life sciences and chemistry. In particular, in the last years polymer-based LoCs have generated a lot of interest because they are low-cost, fast response time, low analyte consumption, disposable devices and suitable for mass production.

The objective of my research is "Femtosecond laser microfabrication technology for the development of disposable polymeric Lab On a Chip". Femtosecond laser technology is a versatile tool for the rapid prototyping of polymeric lab on chip having different application. The fast, precise and contactless laser micromachining technology puts a growing interest in the prototyping of lab on a chip over conventional methods. The ultrashort pulse of femtosecond laser is a unique advantage to direct pattering on materials without any burrs and heat affected zones. This opens a door to improve other current LoC fabrication technologies with different applications. In particular, we are aiming to prototype a Polymeric Lab on chip by exploiting femtosecond laser technology for the purpose of extracting DNA from biological samples (tissues, blood, etc.) and integrate a laser ablated PMMA microdevice into neuroscience research also develop a system of modular microfluidic components that can be combined in a user defined manner.

Two different approaches are established for the fabrication of polymeric LoCs: (i) direct laser ablation of Polymethyl methacrylate (PMMA) substrate to fabricate the fluidic network; (ii) laser cutting of thin polycarbonate (PC) layers to build a multilayer chip structure.

One of the most challenging steps in the fabrication of a microfluidic device is the bonding of a structured substrate with a cover plate to create effectively sealed microchannels. Exploiting the know-how and the laboratory facilities of the industrial partner STMicroelectronics-Lecce, new simple and robust thermally solvent assisted bonding methods were introduced for assembling the polymeric chips. Two procedures are established for PMMA-PMMA (Polymethyl methacrylate) and PC-PC (Polycarbonate) bonding, respectively.

In the First year, two building blocks for the DNA extraction LoC device have designed and optimized the laser micromachining parameters jointly with STMicroelectronics Lecce (Industrial partner). The building blocks are consisting of microfluidic channels, reservoirs, microvalve, inlet and outlet holes. In particular for microvalve a metallic mould is defined.

1. Experimental description

1.1 Experimental setup

Laser micromachining was performed by using the TruMicro 5050 Femto Edition laser. The laser system (Figure 1) provides a laser light of 1030nm wavelength. Laser's pulse duration is 900 fs. The maximum power of the laser light on this system is 40W, as well as the maximum pulse energy is 400µJ.

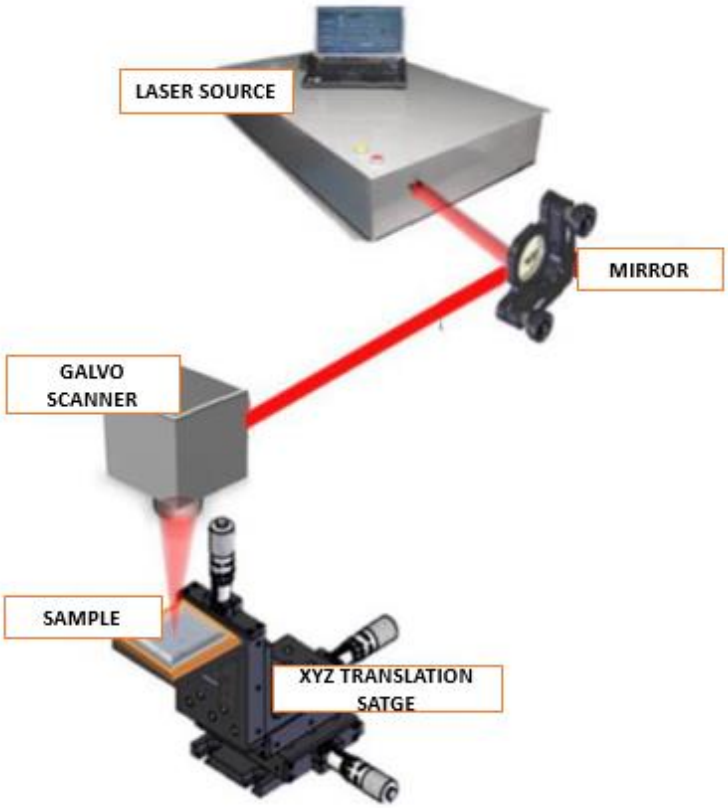

Figure 1: Experimental setup for laser micromachining

The flexibility of ultrafast laser technology and the ability of femtosecond pulses to produce "cold" ablation of the irradiated volume thus avoiding debris and recast layers enables rapid prototyping and high precision micromachining of LoCs devices with complex microfluidic channel networks.

2. Direct Fs laser ablation of PMMA substrate

2.1 T-junction microchannel for DNA extraction LoC

T-junction microchannels are one of the essential parts of a DNA extraction LoC for droplet generation. Manipulating the biological samples in microfluidic devices into droplets instead of a continuous stream requires to separation and detection of the particles.
We fabricated a T-junction micro channel in different dimensions (Tab.1) with two inlet holes and one outlet hole on a 3cmX3cmX1mm PMMA substrate (Figure 2), by exploiting an ultrashort laser source from TruMicro 5050 Femto Edition laser. The laser parameters are listed in (Tab.2).

	Length 'L1' (mm)	Length 'L2' (mm)	Width 'W1' (µm)	Width 'W2' (µm)	Depth 'D' (µm)	Inlet and outlet hole diameter 'd' (mm)
Model 1	4	2	150	100	100	1.8
Model 2	4	2	100	50	100	1.8

Tab 1: Dimensions of T-junction microchannel

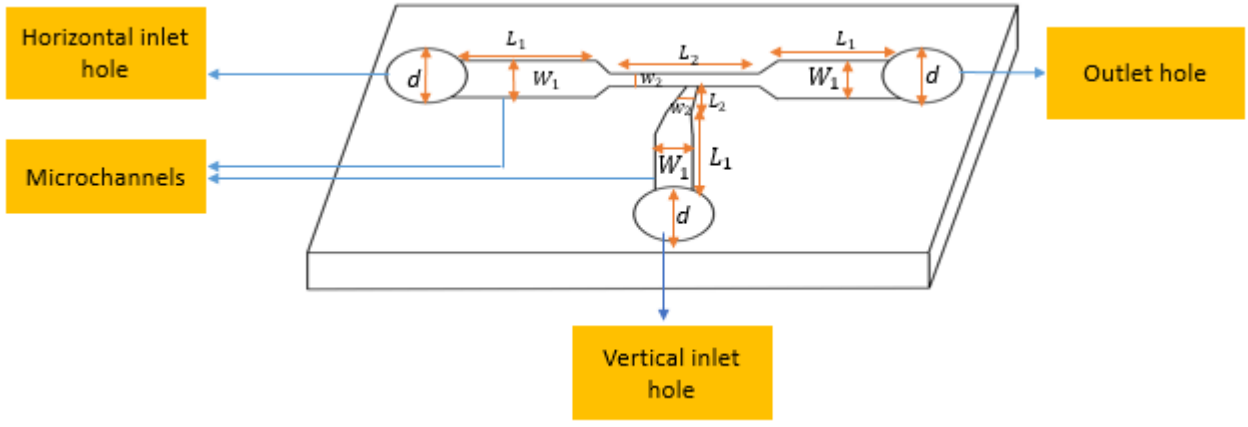

Figure 2: Schematic draw of T-junction microchannel with inlets and outlet

	Microchannel	Inlet and outlet holes
Laser power	0.6W	0.8W
Frequency	50KHz	50KHz
Laser scan speed	40mm/s	25mm/s
Number of loops	1	18
Short pulse energy	12.1µJ	16.1µJ
Hatch distance	5µm	5µm

Tab 2: Laser parameters used for micromachining of T-junction microchannel

2.2 Integration of laser ablated PMMA microdevice into neuroscience research

Neuroscience is the investigations on basic functions of the nervous system for understanding nervous system disorders and medical treatments. Recent developments in miniaturization based on microfabrication helps biologists to overcome the limitations of traditional cell culture methods. Soft-lithography is a widely used method for making micro and nanoscale structures using elastomeric elements and moulds by photolithography. The elastomeric polymer polydimethylsiloxane (PDMS) is the main component of BioMEMS platforms due to its optical transparency, thermal stability, low cost, biocompatibility, gas permeability and ease of fabrication. The devices used for neuroscience research are composed of fluidically isolated culture channels connected by a series of microchannels (Figure 2). This gives more control over the cellular microenvironment, with the ability to create distinct regions to mimic in vivo conditions. Here we are trying to integrate laser ablated PMMA microdevices into the neuronal cell culturing. Furthermore, we tried to fabricate same devices by hot embossing technique.

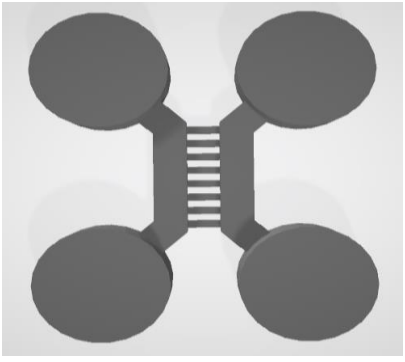

Figure 2: Schematic diagram of a microfluidic device for neuronal cell culture

The laser ablated device was fabricated on a 3cmx3cmx1mm PMMA slab and consists of 2 large culture channels connected by microchannel arrays (Figure 3) 8µm deep and 10 µm wide. The laser parameters used for the fabrication are in the Tab.3

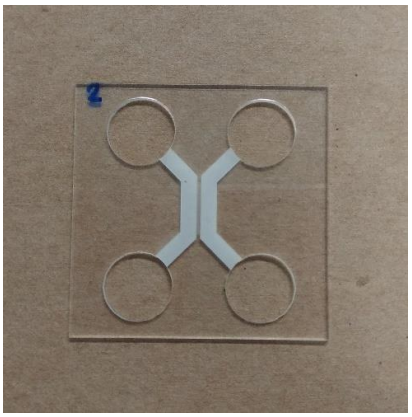

Figure 3: Laser ablated device

	Laser parameters	
	Microcahnnel array	Large culture channel
Frequency	0.625KHz	50KHz
Power	0.010W	0.6w
Laser scan speed	1mm/s	25mm/s
No.of loops	1	1

Tab.3: Laser parameters used for the fabrication of microfluidic device for neuronal cell culture

The device was sealed by a polyolefin tape and the fluid flow was tested with a florescent dyed (calcein) solution, finding that there was any leakage inside the device. But some air bubbles were formed inside the culture channel due to the relatively low depth of the well with consequent verylow amount of fluid filling it (Figure 4).

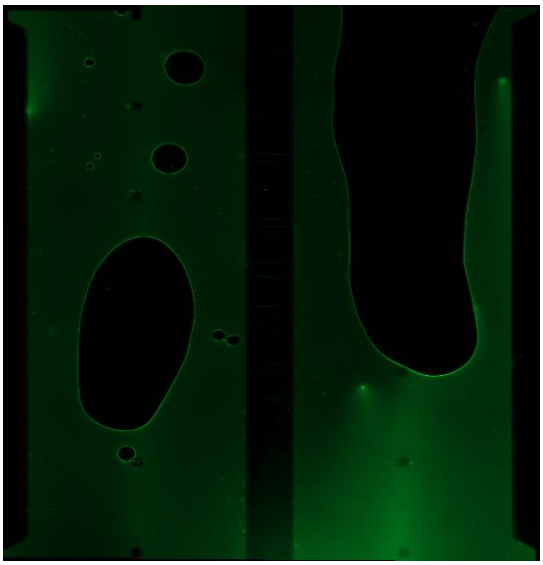

Figure 4: Microscopic image of the fluid flow (fluorescent dye solution) inside the device

Hot embossing:

Hot embossing is fundamentally the stamping of a pattern into a polymer softened by raising the temperature of the polymer just above its glass transition temperature. We stamped a micropattern on 5mm thickened PMMA (Figure 5) relying on hot embossing protocol with the substrates made by photolithographic method (silicon substrate and UV glue substrate). The parameters used for hot embossing are in the Tab.4

Loading temperature	**120°C**
Loading time	15 min
Cooling temperature	22°C
Cooling time	1 hour
Pressure load	0.2 ton

Tab.4: Parameters used for hot embossing

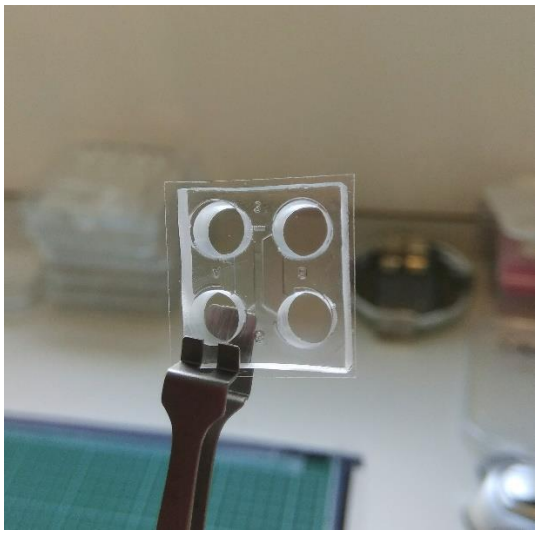

Figure 5: Hot embossed micropattern on a 5mm thickened PMMA substrate

The hot embossed device showed fluid leakage through the edge of the inlet holes after testing with calcien.

2.3 Modular microfluidic system consisting laser ablated microchannel

Modularity ultimately allows a researcher to purchase premade components and build their own network of devices, allowing them to design bespoke assays and experiments for a wide range of different studies, according to their needs.

Press fitting PDMS blocks into an enclosure of PMMA is a new idea raised to overcome the fluid leakage while joining PDMS blocks manufactured on 3D printed moulds. 3D printed mould shows irregularities on side walls which is enough for fluid leakage. A 15x15mm size enclosure has been made from PMMA, with CNC machined holes. Between each hole, 100µm deep channels have been laser ablated to connect the separate PDMS blocks (Figure 6).

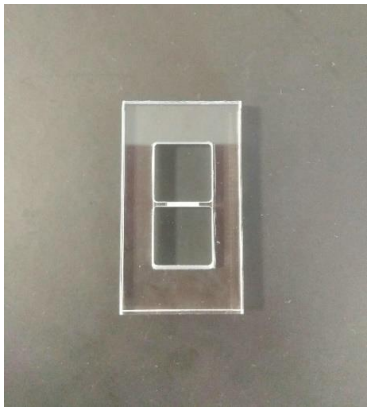

Figure 6: PMMA enclosure for press fitting the PDMS blocks

The press fitted PDMS block device has been sealed with polyolefin tape. The fluid flow test performed using calcein showed fluid leakage at the lateral interface (Figure 7).

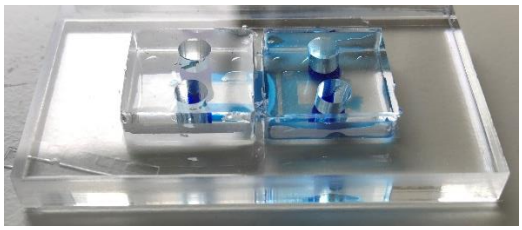

Figure 7: Fluid leakage through the lateral interface of the press fitted PDMS block device

3. Fs laser cutting of thin polycarbonate sheet

3.1 Multilayer chip

Multilayer chip is another approach of prototyping a polymeric lab on a chip. The microfluidic system is produced with layer-by-layer manufacturing technology of laser-cut thin polymer sheets. Direct processing with ultrashort pulses offers a great potential for rapid prototyping together with possibility of getting smaller and more precise structures. A 250-micrometer polycarbonate (PC) shows desired material properties for a microfluidic system (hydrophobic, hydrophilic, transparent, etc.). Initially, the microfluidic system is constructively divided into individual layers (Figure 8). The microstructures on each layer are formed separately by laser cutting of thin PC (Figure 9). The microstructures are decided based on the final function and design of the LOC. Finally, all separate layers are stack together and joined to form a single chip by using different bonding techniques. We chose a rapid solvent bonding in between the layers. The parameters used for the laser cut given in Tab.5

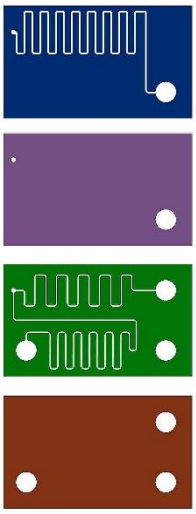

Figure 8: Schematic diagram of individual layers of multilayer chip

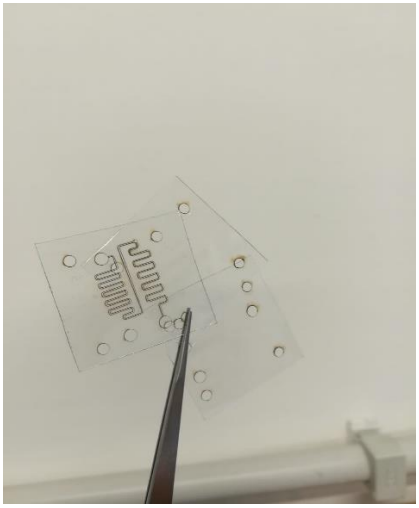

Figure 9: Laser cut microstructures on individual layers

Laser parameters	
Frequency	50KHz
Power	0.4W
Laser scan speed	40mm/s
Short pulse energy	8.1µJ
Number of loops	10
Hatch distance	5µm

Tab.5: Parameters used for laser cut

4. Bonding of microfluidic devices

Bonding is the final step of microfabrication and one of the most important steps to manufacture microdevices. Effectively sealed micro structured substrate is essential for the perfect functioning of microdevices. Thus, this process plays a key role in the manufacturing process of microdevices. Various bonding techniques have been developed for thermoplastic materials, such as thermal fusion bonding, chemical bonding, and solvent bonding.

4.1 PMMA-PMMA bonding

Conventional methods to bond PMMA to PMMA are thermal bonding UV assistant bonding, solvent bonding, microwave bonding, and friction spot welding. Here a cheap, deformation-free, and very simple method to bond PMMA-PMMA substrates is introduced which uses low pressure and moderate temperature in less time. This method is based on pouring isopropyl alcohol (IPA) between two PMMA substrates at moderate temperature.

PMMA cannot be dissolved in IPA at room temperature because of the difference in the solubility parameter of PMMA with IPA. The solubility parameter of PMMA is 20$(Jcm^{-2})^{1/2}$ and of IPA is 23.4 $(Jcm^{-2})^{1/2}$.

As a first step of bonding, the PMMA substrate was cleaned with IPA to eliminate the dust on the surface of PMMA. Nitrogen gas was used to dry out the substrates at room temperature. A few drops of IPA were poured onto the patterned surface of PMMA which was then covered with another plane PMMA slab. Then both PMMA slabs were stacked together with a plastic clamp (Figure 10). Finally the sample was put into a preheated oven at 120˚C for 5 minutes to get the chip bonded (Figure 11,12). A fluid injector portal was fixed at the inlet to inject the fluid inside (Figure 13). The fluid flow was tested by pumping water into the microchannel by using a micropump.

Figure 10: Stacked PMMA slabs with plastic clamp

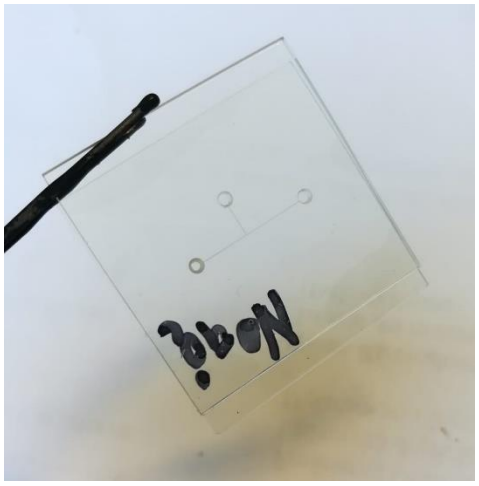

Figure 11: Bonded chip

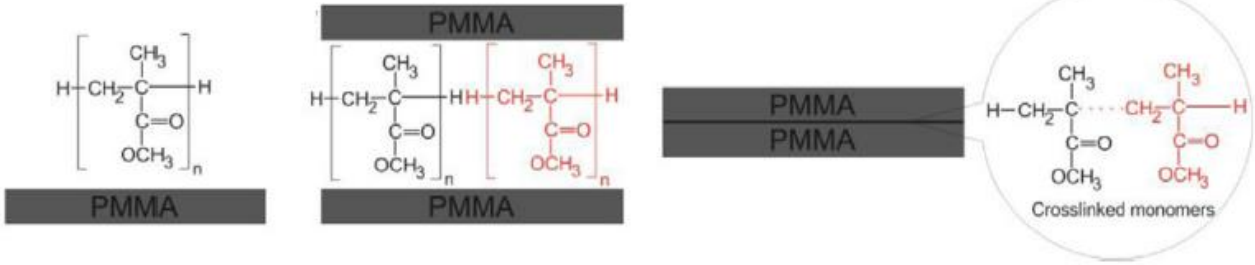

Figure 12: Chemical reaction on the surface of the PMMA substrates

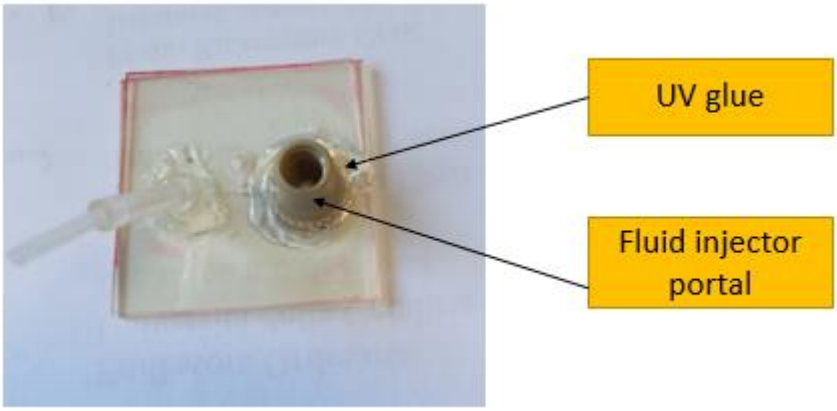

Figure 13: Fluid injector portal at inlet to inject the fluid

4.2 PC-PC bonding

A one step solvent bonding method was introduced for polycarbonate (PC), too. This fast solvent bonding was realized by using acetone and pentane mixture solution at 3:7 ratio. PC is highly soluble in pure acetone at room temperature. n-pentane, a solvent with a boiling point of 36.1 °C, acted as a sacrificial solvent and acetone acted as the solvating solvent, as acetone's boiling point is 56.1 °C. PC is almost insoluble in n-pentane. The solution was poured between all the layers to be bonded. All the layers were hitched up together in the manner of final chip. Finally, the samples were put on a hot plate for 15s at 60˚C. The difference in the boiling point made n-pentane evaporate faster than acetone, which drastically increased the acetone concentration. The PC polymer chains became entangled with those on the two substrates, which led to a strong bonding (Figure 14) and all the layers bonded each other to become a single chip.

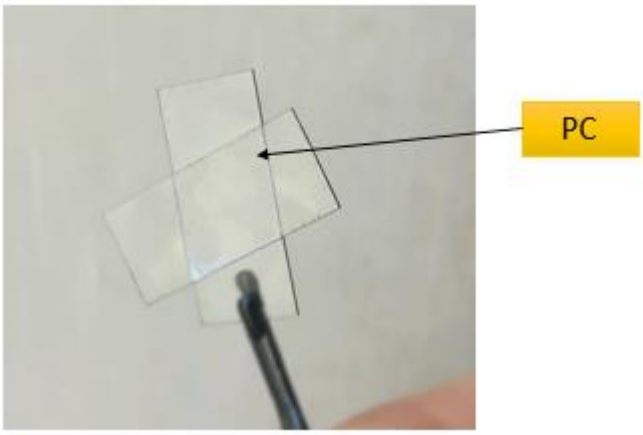

Figure 14: Example for bonded PC layers

Future work

The next step of integration of laser ablated PMMA into microfluidics for neuroscience research is to try neuronal cell culture on a 5mm thickened laser ablated PMMA device and testing press fitting PDMS blocks enclosure system with CNC machined PMMA moulds.

During the 3rd year the activity will aim at manufacturing, by exploiting the femtosecond laser technology, and assembling all the building blocks of a polymeric lab on chip that can extract DNA from biological samples (blood, tissues, etc,). The final device will be validated at the end of the PhD project.

Publications

1. "Prediction model of the depth of the femtosecond laser micro-milling of PMMA" (Accepted in Optics&Laser Technology journal)

Poster presentation

1. "Fs-laser based smart procedures for the fabrication of polymeric Lab on a Chip devices" – Science and Industry for environment, Health and Digital Society Technologies; Industrial PhD Day at Università degli Studi di Bari Aldo Moro – 26 June 2019

Summer school

1. International School on Laser Micro/Nanostructuring and Surface Tribology 1-5 October 2018 – Bari, Italy
   "Femtosecond laser micro-fabrication of polymeric lab-on-chip for advanced and mini-invasive diagnostics" – Oral presentation