Dipartimento Interateneo di Fisica "*Michelangelo Merlin*"

Dottorato di Ricerca in Fisica XXXII ciclo

PhD: Udith Krishnan

1st year activity report

Course work:

The training activities carried out during the 1st year is in an interdisciplinary way. One course stands for improving language skills, one another gives a knowledge about European research model and promotion of research results. 2 courses helps to gather a basic programming grip. Other 3 subjects are more specific to concerning my research activity. The courses I am following and going to do are summarized in the below table.

Course	Professor	Period	Hours	CFU	Final test
Management and knowledge of European research model and promotion of research results	D'Orazio	June	16	2	Attestato frequenza
How to prepare a technical speech in English	White	April-May	16	2	Oral presentation
Fundamentals in advanced programming using C++ programming language	Cafagna	June-July	22	2	Final test
Interpolation Methods e techniques for Experimental Data Analysis	Pompili	September-October		2	Final test
Introduction to parallel Computing and GPU Programming using CUDA	Pantaleo	June	16	2	Final test
Fluidodinamica computazionale	Pascazio Giuseppe	September-October		4	Final test
Optical sensors and spectroscopic tecniques	Spagnolo/ Patimisco	June-July	20	2	Final test
Total				16	

Research activity:

The objective of my research is to design, implement and validate a prototype of polymeric Lab On a Chip (LOC) that can extract DNA from a biological sample, by Femtosecond laser technology for rapid prototyping and LoCs fabrication. The materials traditionally used for the manufacture of LoCs are silicon and glass, but to produce low-priced pre-sterilized and usable in any environment we have to pass to polymeric materials. Among the various polymers, polymethylmethacrylate (PMMA) has excellent mechanical, optical and chemical properties.

A Lab On a Chip (LOC) can be defined as a device in which multiple laboratory techniques are integrated in a chip with a footprint of at most a few tens of square centimetres. LoCs have tremendous potential for application in various fields of life sciences and chemistry. 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.

The device that is to be developed will have the purpose of extracting DNA from biological samples (tissues, blood, etc.). Integrating the DNA extraction into a single chip would have the twofold advantage of increasing device portability and reducing the amount of sample used. DNA detection is crucial in many fields, including clinical and veterinary diagnostics, industrial and environmental monitoring, agricultural research and forensic science. Disease diagnosis and prognosis are based on the effective detection of disease status (Eg: cancer), infectious organisms (Eg: HIV), and genetic markers.

In the First year, two building blocks to the device for DNA extraction is designed jointly with STMicroelectronics Lecce. The building block consist of microfluidic channels, reservoirs, microvalve, inlet and outlet holes. In particular for microvalve a metallic mould is defined.

1st building block:

We introduced the fundamental building blocks of a microfluidic device. The basic building blocks that is, microvalve housing, microchannel, square shaped micro chamber, inlet and outlet holes. Based on this, made a whole design by combining all the blocks by means of a software named TruTops (figure 1). By focused on to maintain the same depth for each blocks we fixed the laser parameters, despite we choose alternating direction lines hatching. Laser micromachining was performed by using the TruMicro 5050 Femto Edition laser. The laser system 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.

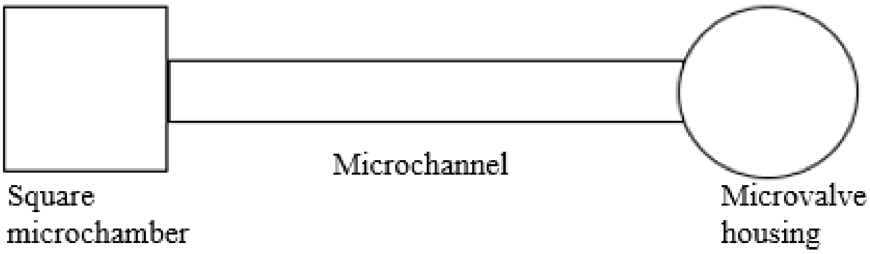


Figure 1

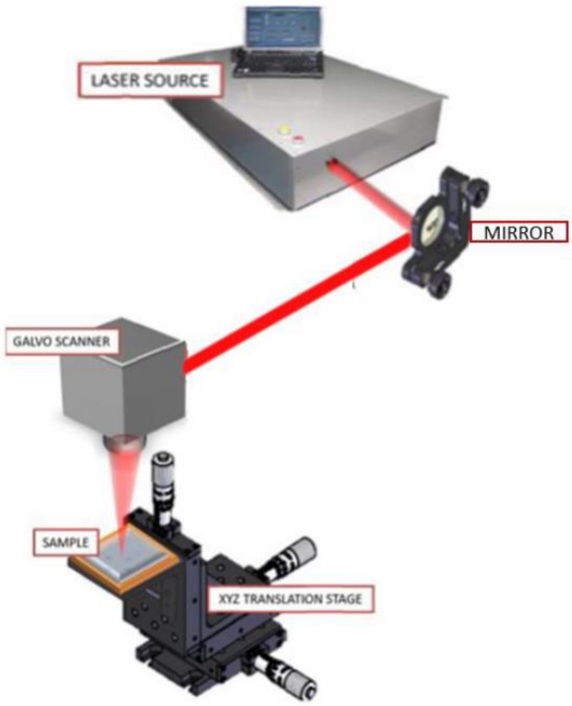


Figure 2: schematic representation of laser micromachining setup

The laser parameters for each block is given below.

	Microchannel	Microvalve housing	Square micro chamber
Laser parameters	Values		
Short pulse energy	14.1µJ	14.1µJ	14.1µJ
Laser scan speed	15 mm/s	25 mm/s	25 mm/s
Number of loops	3	2	2
Frequency	100 KHz	100 KHz	100 KHz

These parameters are used to optimized the depth for square microchamber is 128µm (figure 3), microchannel is 130µm (figure 4) and microvalve housing is 129µm (figure 5) on a square (3x3x1 cm^3) PMMA slab.

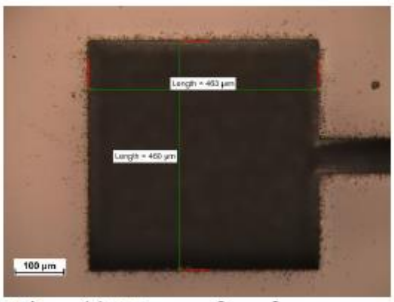


Figure (a) : Upper surface of square microchamber on PMMA

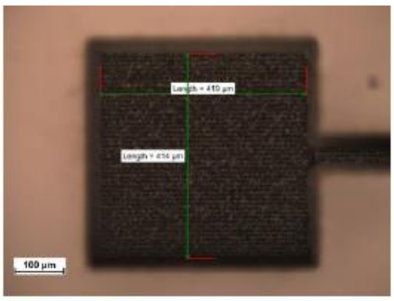


Figure (b) : Bottom surface of square microchamber on PMMA

Figure 3

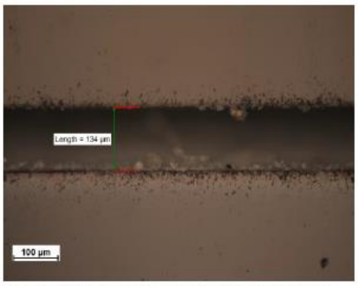


Figure (a): Upper part width of microchannel (134µm) on PMMA substrate

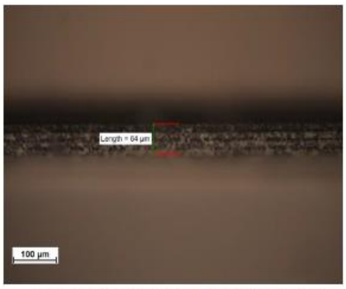


Figure (b) : Bottom part width of micro-channel (64µm) on PMMA substrate

Figure 4

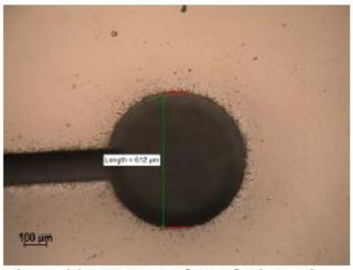


Figure (a) : Upper surface of microvalve housing(diameter-612µm) on PMMA

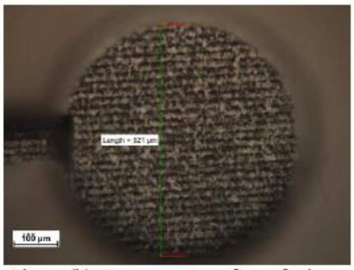


Figure (b): Bottom part surface of micro-valve housing(diameter-521µm) on PMMA

Figure 5

Subsequently, we drilled 2 holes with 38µm radius from the other side of the slab (figure 6). One hole is on the square chamber and the other one is on microvalve housing which stands for inlet and outlet. The laser parameters for hole drilling is given below.

Laser parameters	Values
Short pulse energy	37.1µJ
Frequency	100KHz
Laser scan speed	10 mm/s
Number of loops	5

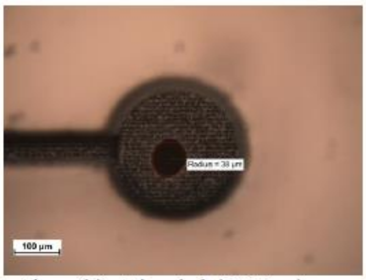


Figure (a) : Micro-hole(R=38µm) on microvalve chamber

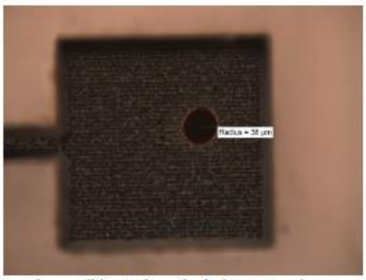


Figure (b) : Micro-hole(R=38µm) on square microchamber

Figure 6

The tube we have for pumping the fluid into the chip has a diameter of 800µm. Therefore, we introduced another layer of PMMA substrate with two holes of 1mm (figure 7) diameter exactly over the top of inlet and outlet holes.

Figure (a): Upper diameter(1034µm) of larger hole on the next layer of PMMA

Figure (b) : Bottom diameter(744µm) of larger hole on the next layer of PMMA

Figure 7

<u>Mould for microvalve on metals:</u>

The mould for microvalve is fabricated on metallic plate. ST Microelectronics, the industrial partners of this project have a prototype of valve in millimeter dimensions (figure8). We miniaturize the dimensions from millimeters to micrometers and fixed the expected dimensions as upper diameter of 590µm and bottom 230µm with a depth of 135µm. We fabricated a groove structure (figure 9) with 18 loops having lines hatching from 590µm to 130µm in a manner of decreasing the diameter of each loop with 4.6µm from upper diameter to bottom diameter.

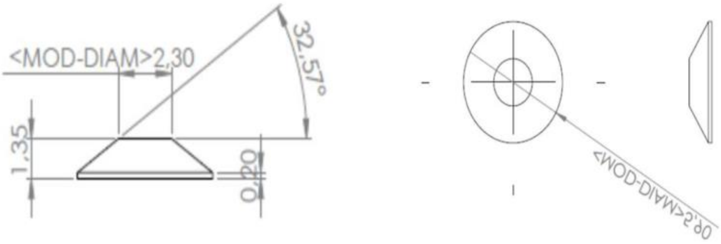


Figure 8: Schematic diagram of prototype of valve in millimeter dimension

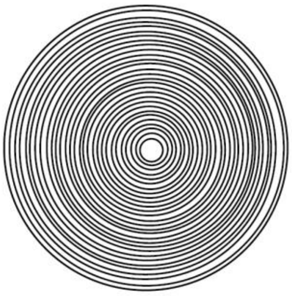


Figure 9: Groove structure

Parameters	Values
Short pulse energy	8.9µJ
Laser scan speed	350mm/s
Laser power	0.9W
Frequency	100KHz
Number of loops	18

The parameters defined in the above table led us to fabricate the desired geometry with an upper diameter of 596µm, bottom diameter of 230µm and depth of 133.8µm with a uniform surface at the bottom surface (figure 10).

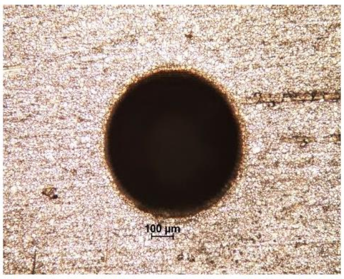


Figure (a) : Upper diameter(596µm) of microvalve with Lines hatch style

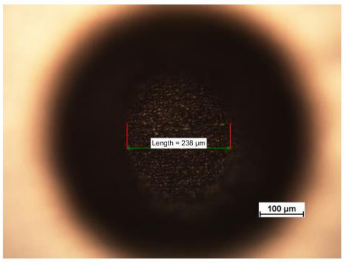
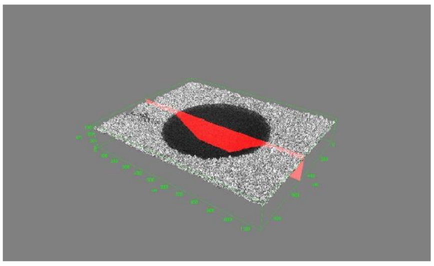
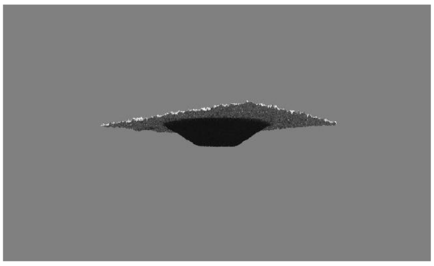


Figure (b) : Bottom diameter(238µm) of microvalve with Lines hatch style

Figure 10



(a)



(b)

Figure 11: Confocal microscopic images of microvalve

In addition to we fabricated a mould for valve in millimeter dimension by increasing all the dimensions of microvalve upto 10 times, on a metallic workpiece of thickness 2mm, despite the procedure. The laser parameters used for this micromachining is given below.

Laser parameters	Values
Short pulse energy	8.6µJ
Frequency	100KHz
Laser power	0.9W
Laser scan speed	350 mm/s
Number of loops	159

We obtained a valve with upper diameter of 6mm, bottom diameter of 2.34mm (2347µm) and a depth of 1.29mm (1296µm) with a uniform bottom surface (figure 12).

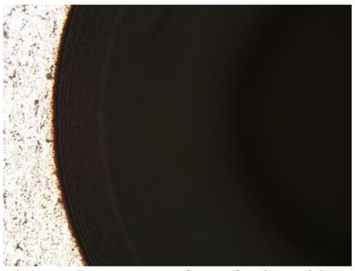


Figure (a) : Upper surface of valve with millimeter dimension (D=6mm)

Figure (b) : Bottom diameter(2347µm) of valve with millimeter dimension

Figure 12

2nd building block:

We introduced another building block consist of microchannel , 3 holes and 2 inlet and outlet by exploiting a ultrashort laser source from Active Fiber Systems GmbH for micromachining(figure 13). The whole building block fabricated in a manner of combination of 2 PMMA slabs (figure 16). One slab consist of microchannel, inlet and outlet holes and a straight hole from the opposite phase of PMMA substrate into the centre of microchannel for inserting the fiber (figure 14). The other slab has 2 inclined holes with 30˚ from the straight hole (figure 15). The inclined holes contrived by using a 360˚ rotating stand.

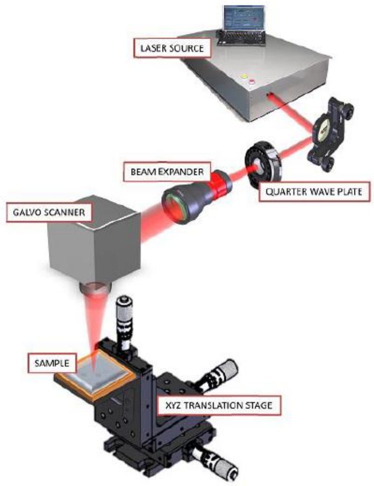


Figure 13: Schematic representation of laser micromachining setup

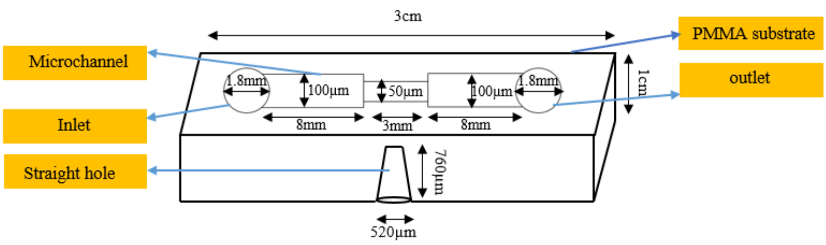


Figure 14: 3D Schematic representation of PMMA slab consist of microchannel, straight hole, inlet and outlet

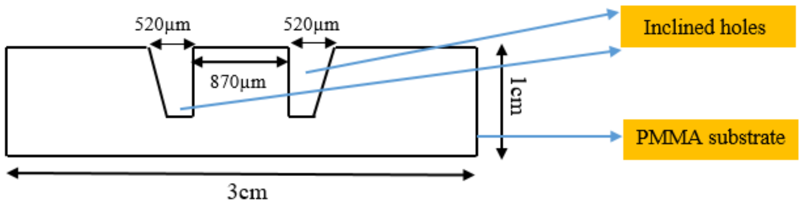


Figure 15: 2D The PMMA slab comprise 2 inclined holes with 30˚ from the straight hole

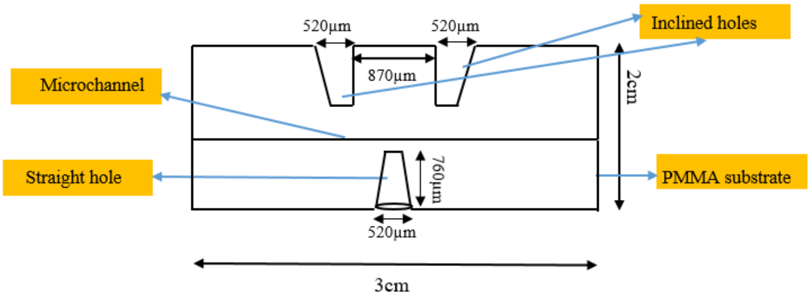
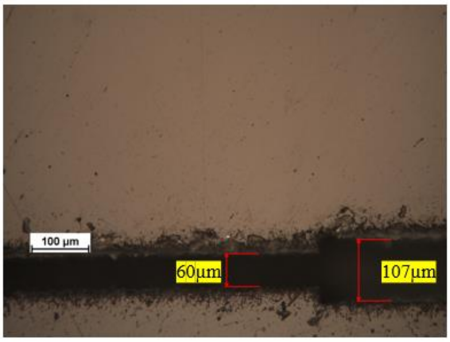


Figure 16: 2D schematic representation concerning Combination of the 2 PMMA slabs

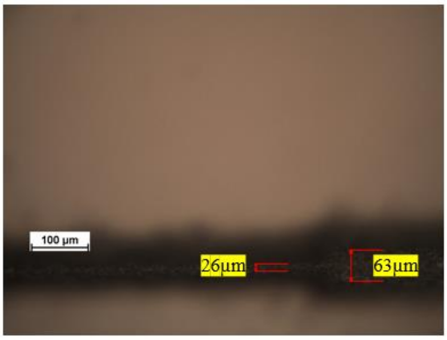
The fiber laser system we used to be with a ultraviolet pulses (pulse duration 650-fs) working at a wavelength of 1030 nm at a frequency of 50 KHz and maximum pulse energy of 100µJ (or maximum average power of 50 W).

	Microchannel	Holes for inserting fiber	Inlet and outlet holes
Laser parameters	values		
Laser power	0.5W	0.6W	2.5W
Frequency	50KHz	50KHz	50KHz
Laser scan speed	20mm/s	20mm/s	200mm/s
Number of loops	1	10	20

By using the above mentioned laser parameters we fabricated a microchannel with a depth of 53µm (figure 17), 3 holes for inserting the fiber that is straight hole with a depth of 761µm (figure 18) and inclined holes have 750µm of depth (figure 19). Furthermore 2 inlet and outlet holes to the chip (figure 20).

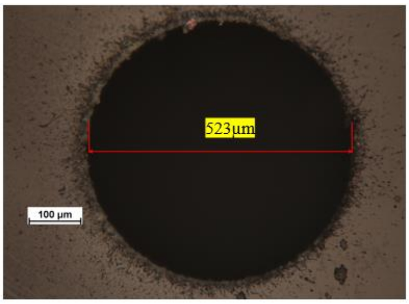


Figure(a): Upper part width microchannel on PMMA substrate

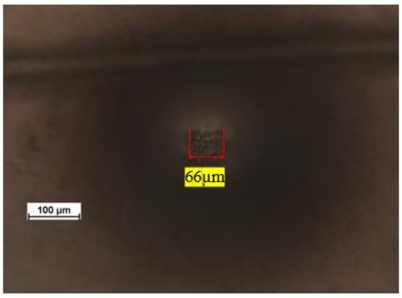


Figure(b): Inner part width of microchannel on PMMA substrate

Figure 17



Figure(a): Upper diameter of straight hole



Figure(b): Inner diameter of straight hole

Figure 18

Figure(a): Upper diameter of 30˚ inclined hole

Figure(b): Inner diameter of 30˚ inclined hole

Figure 19

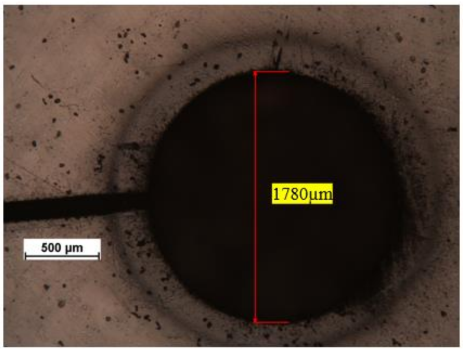


Figure 20: Upper diameter of inlet and outlet hole

<u>Future work:</u>

During the second year it is expected that the first prototype of LoCs devices will be manufacture in PMMA for DNA extraction. In this phase I will study how to implement the microvalves, micro pumps and other accessories needed for the perfect functioning of the devices in the prototypes. In the second year there is a 6 - month research activity period with 14th group of coordinated research by Dr Michele Zagnoni at University of Strathclyde in Glasgow (UK), during which I will focus on optimizing the design and testing of the first chips with biological samples.

The THIRD and final year of activity will be dedicated to the manufacture, assembly and validation of the final device. Specifically, the validation and testing phase, with an estimated duration of 6 months, will be held at the STMicroelectronics laboratory in Lecce under the supervision of the company tutor.