UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Departimento Interateneo Di Fisica "M. Merlin"

Dottorato di Ricerca in Fisica XXXII ciclo

Femtosecond laser microfabrication technology for the development of disposable polymeric Lab On a Chip

Industrial PhD with ST Microelectronics Lecce

(Tutor: Ing. Francesco Ferrara)

2nd year activity report

PhD student: Udith Krishnan

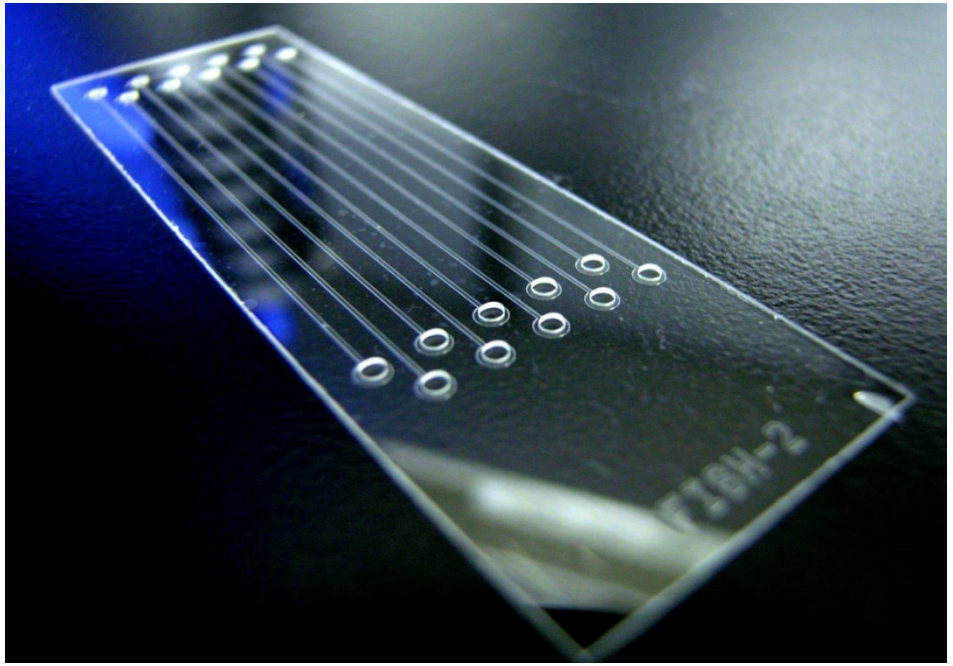
Tutor : Dr. Antonio Ancona

Outline

- Introduction
- Polymeric lab on a chip
- Femtosecond laser technology for lab on a chip(LOC)
- Aim of the research
- Experimental setup
- Direct Femtosecond(Fs) laser ablation of PMMA substrate
- Femtosecond(Fs) laser cutting of thin polycarbonate(PC) sheet
- Bonding of microfluidic devices
- Future work

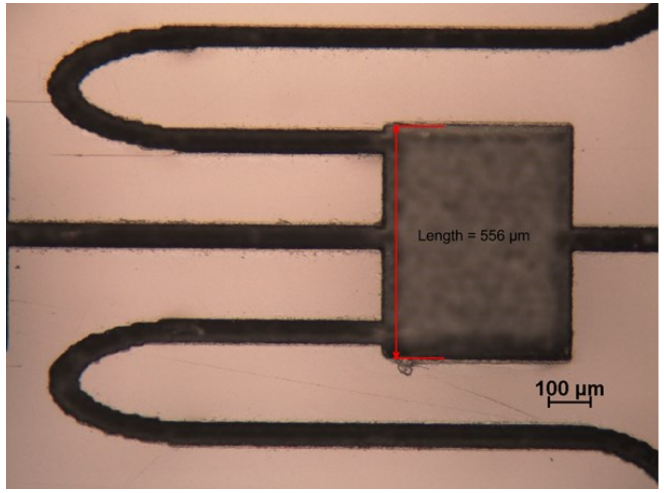
Polymeric lab on a chip

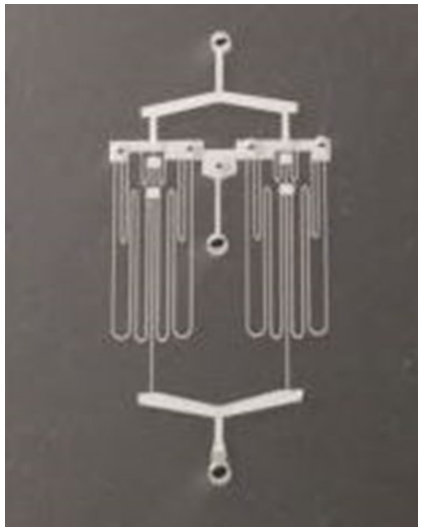
- Previously used materials: silicon and glass
- Polymeric materials
- Polymethylmethacrylate(PMMA)
- Advantages: excellent mechanical, chemical, optical properties



Femtosecond laser technology for lab on a chip

- Rapid prototyping by direct laser ablation
- Micrometric precision
- Possibility of sealing the channel by direct fs laser bonding of transparent polymers
- Cold ablation





Aim of the research

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

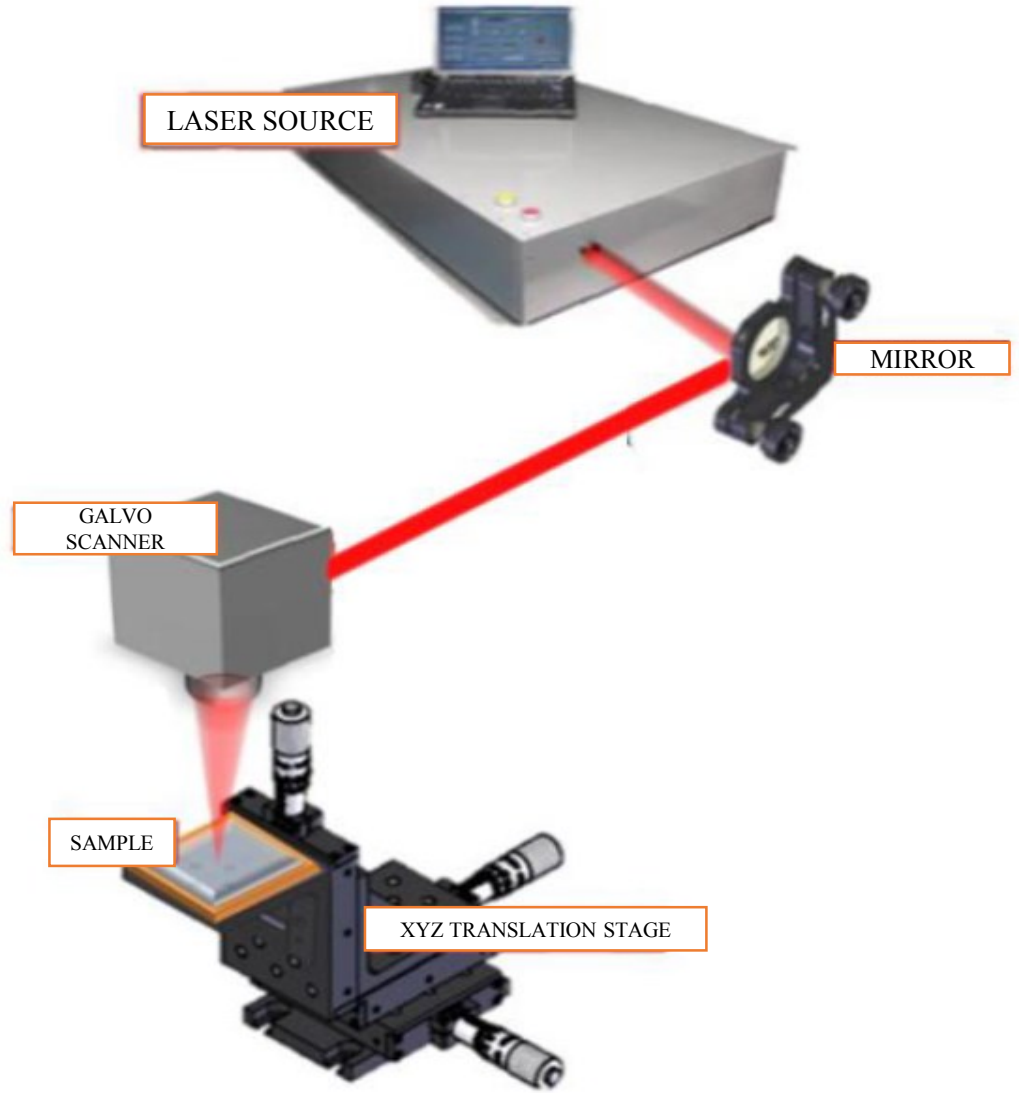
❑ Femtosecond laser microfabrication technology for the development of disposable polymeric Lab on a Chip

A. Prototype a polymeric lab on a chip for the purpose of extracting DNA from biological samples

B. Integration of laser ablated PMMA microdevice into neuroscience research

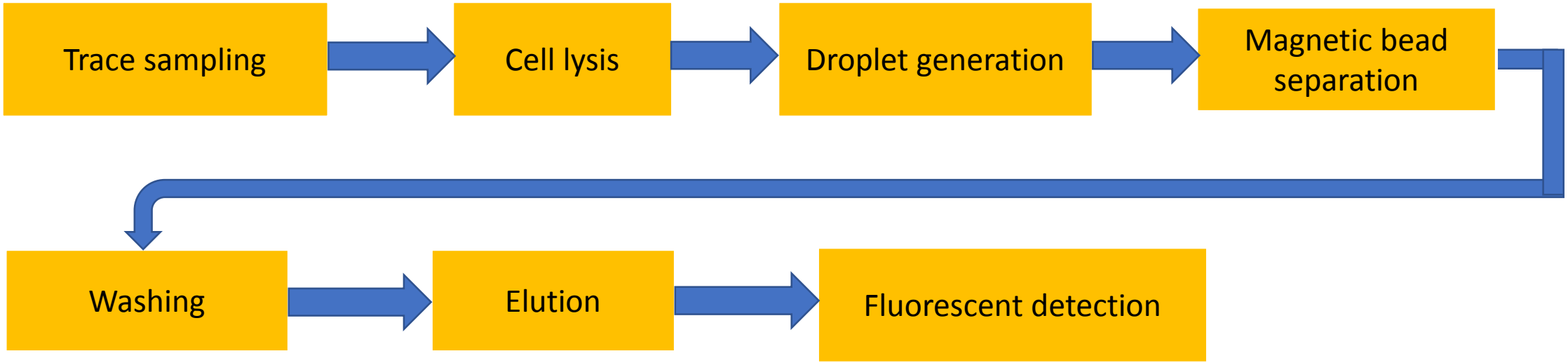
   (a) develop a system of modular microfluidic components that can be combined in a user defined manner

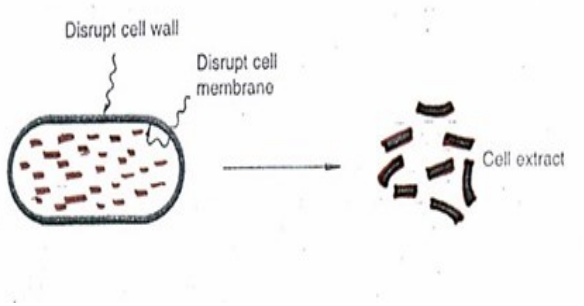
Experimental setup



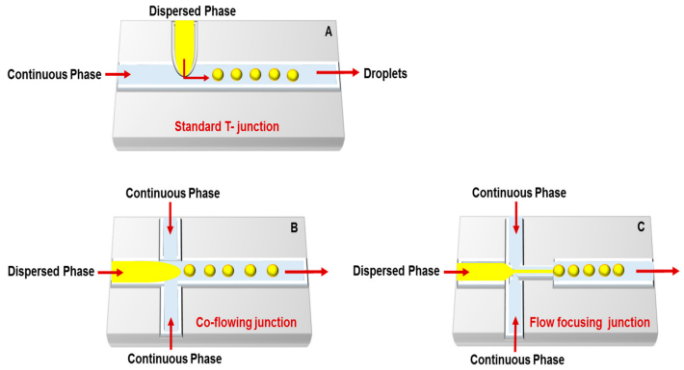
Laser system : TruMicro 5050
Femto Edition laser
Wavelength = 1030nm
Pulse duration = 900fs
Max. Power = 40W
Max. Pulse energy = 400µJ

Example of DNA extraction processing steps

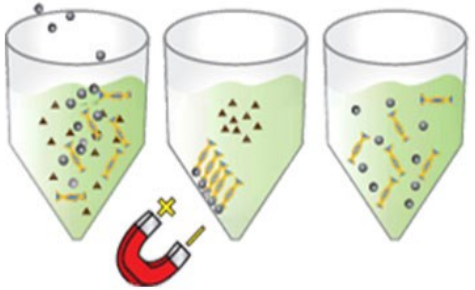




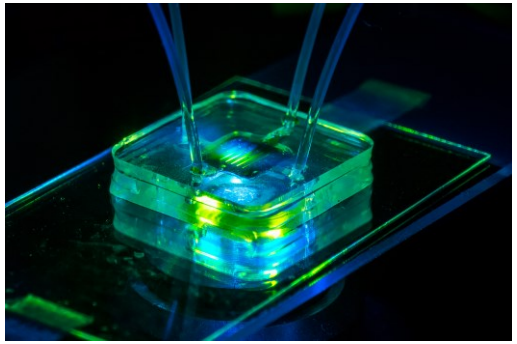
Cell lysis



T junction droplet generation



Magnetic bead separation



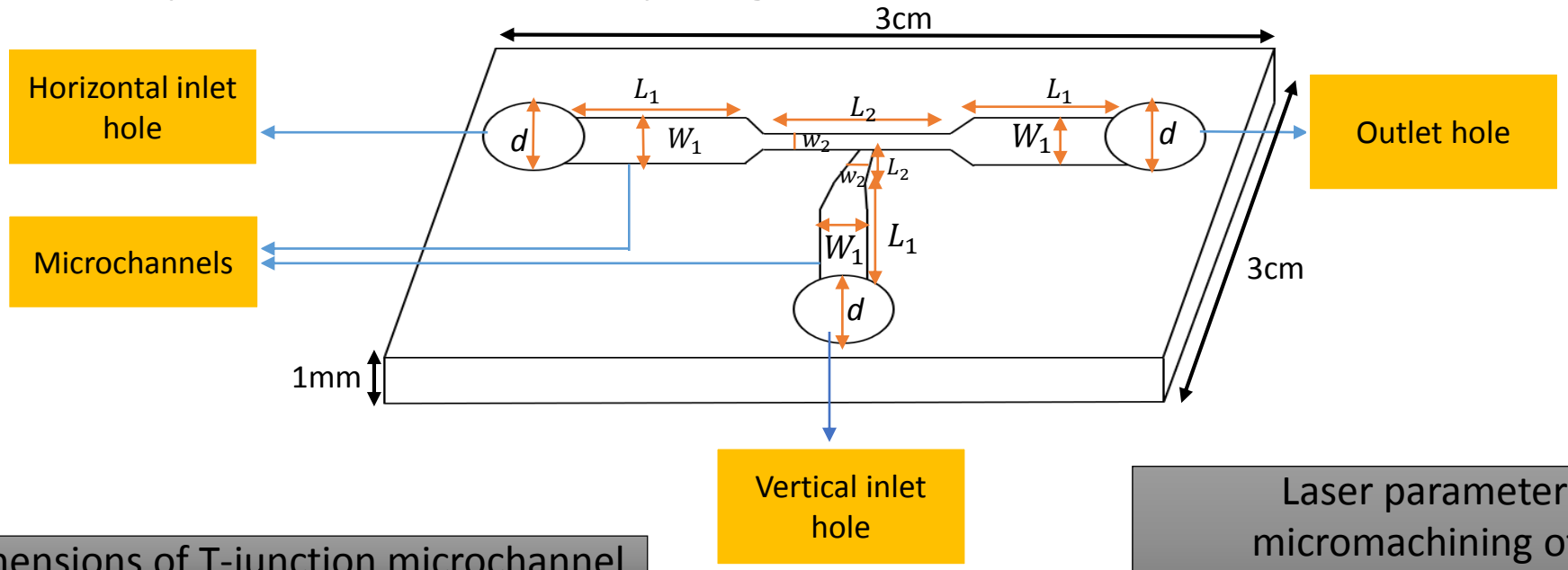
Fluorescent detection

Direct Fs laser ablation of PMMA substrate

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

1. T-junction microchannel for DNA extraction LoC

- Essential part of a LOC for droplet generation



Dimensions of T-junction microchannel

	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

Laser parameters used for micromachining of T-junction microchannel

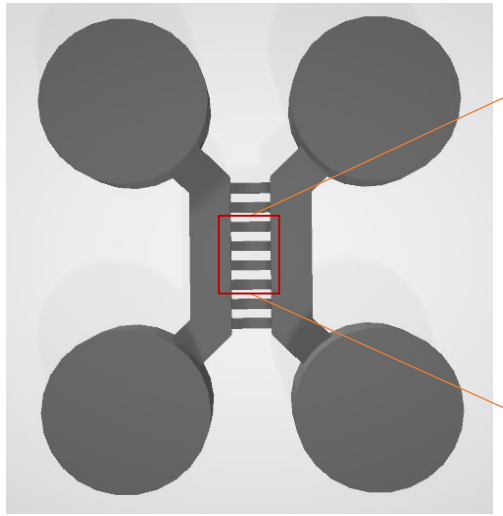
	Microchannel	Inlet and outlet holes
Laser power	0.6W	0.8W
Frequency	50KHz	50KHz
Laser scan speed	40mm/s	25mm/s
Number of loops	1	18
Short pulse energy	12.1µJ	16.1µJ
Hatch distance	5µm	5µm

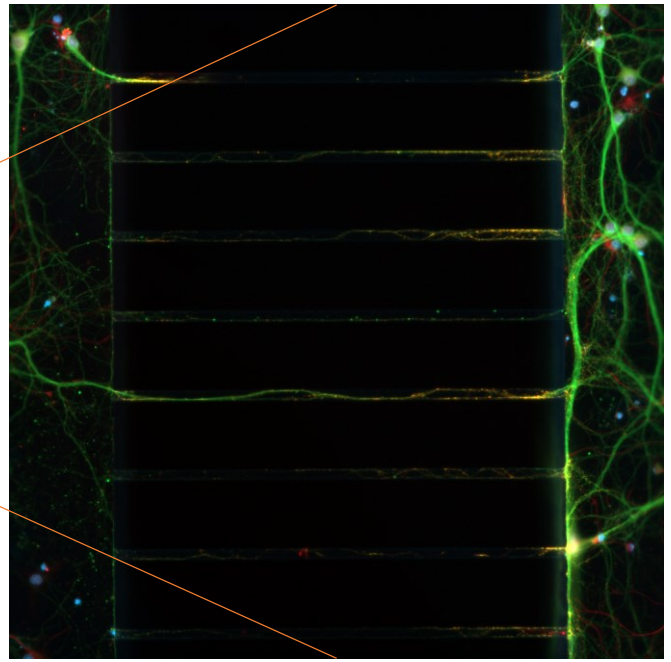
Direct Fs laser ablation of PMMA substrate

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

2. Integration of laser ablated PMMA microdevice into neuroscience research

- Neuroscience investigates the basic functions of the nervous system for understanding nervous system disorders and medical treatments
- Soft lithography is widely used method
- Conventionally used material is PDMS
- Device composed of fluidically isolated culture channels connected by a series of microchannels
- Gives more control over the cellular microenvironment, with the ability to create distinct regions to mimic in vivo conditions.
- Culture different cell types in different compartments



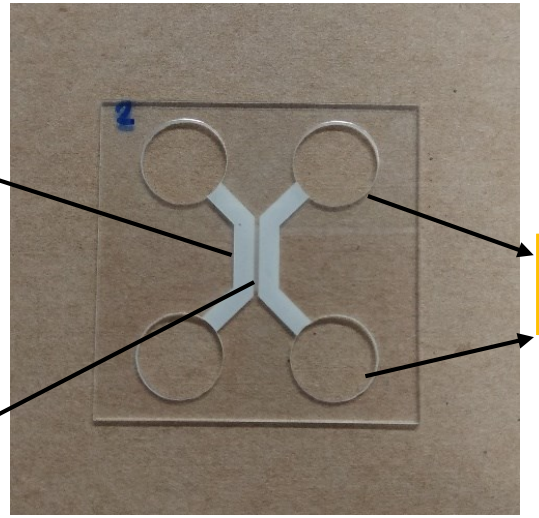


Direct Fs laser ablation of PMMA substrate

2. Integration of laser ablated PMMA microdevice into neuroscience research

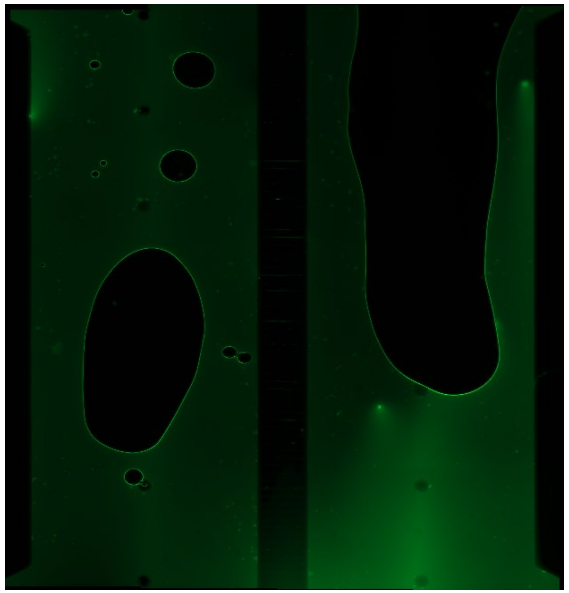
2mm wide and 100µm deep channel

8µm deep and 10µm wide microchannels



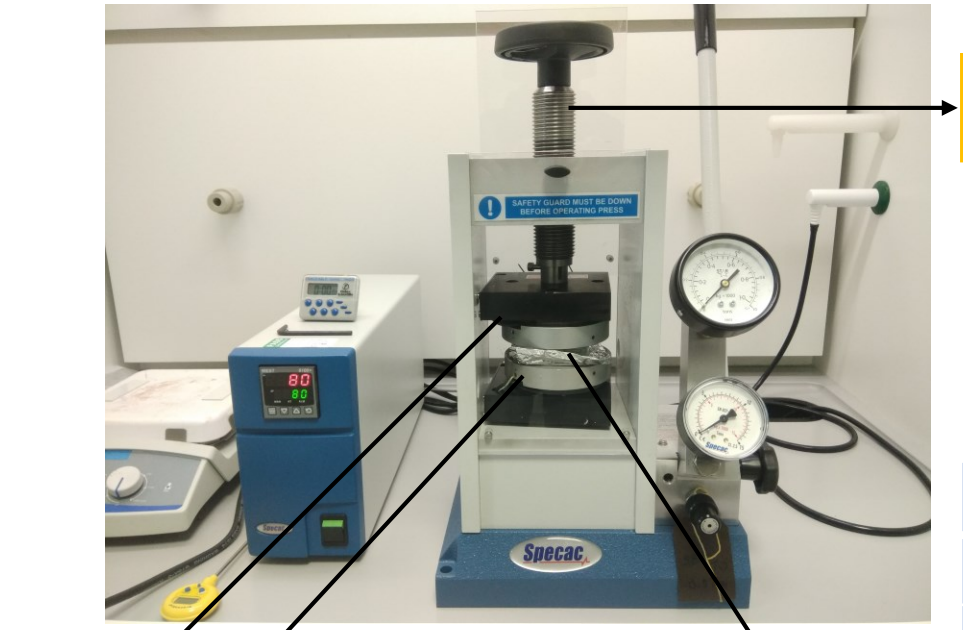
8mm well

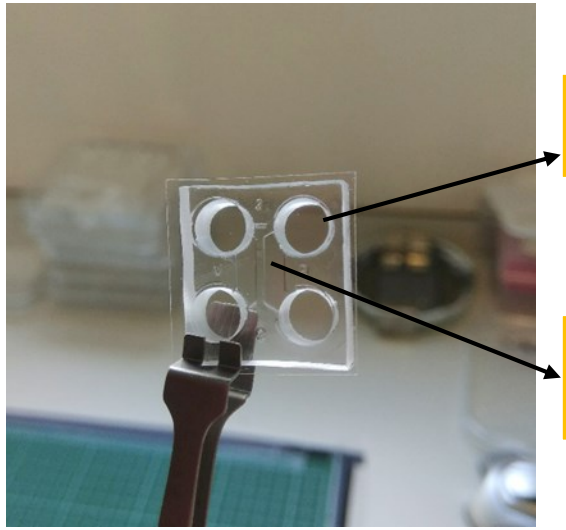
	Laser parameters	
	Microcahnnel array	Large culture channel
Frequency	0.625KHz	50KHz
Power	0.010W	0.6w
Laser scan speed	1mm/s	25mm/s
No.of loops	1	1



- Sealed with polyolefin
- Tested by calcein
- No leakage

- **Hot embossing technique on PMMA**
- Stamping of a pattern into a polymer softened by raising the temperature of the polymer just above its glass transition temperature
- Silicon and UV glue substrates were used





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

- Sealed with polyolefin
- Leakage found through the edges of holes
- Surface deformation of sample could also be a problem

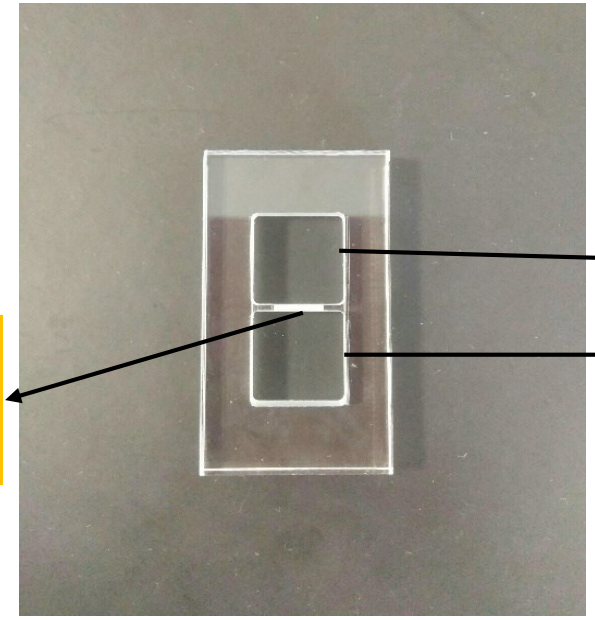
Direct Fs laser ablation of PMMA substrate

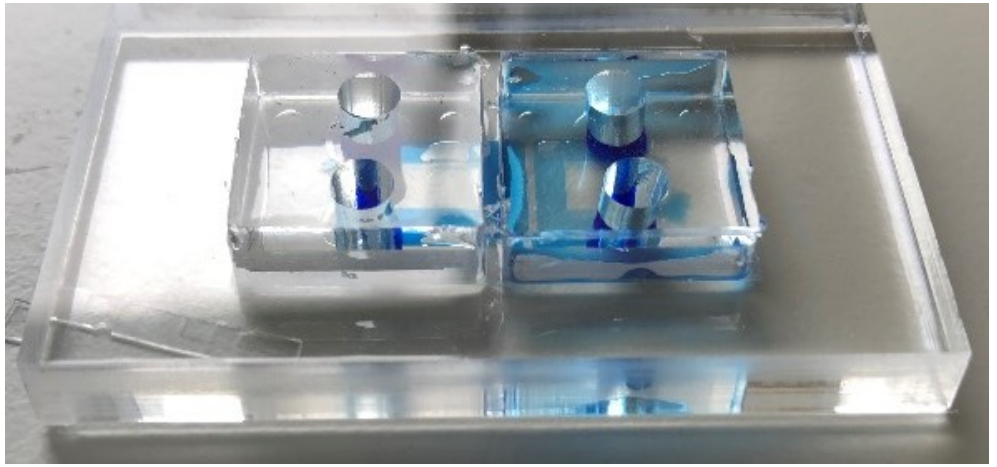
3. Modular microfluidic system consisting of laser ablated microchannels

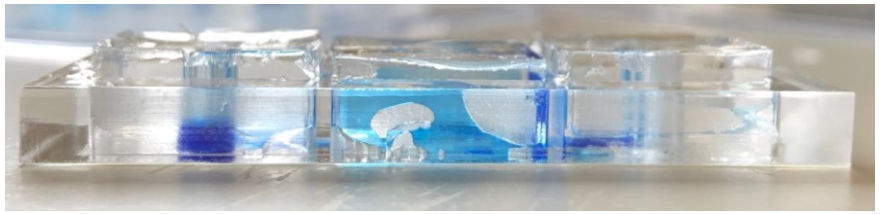
- Modularity is attractive for non-technical users and would allow reconfiguration
- Allow a researcher to purchase premade components and build their own network of devices

Press fitting PDMS blocks into an enclosure of PMMA

- 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







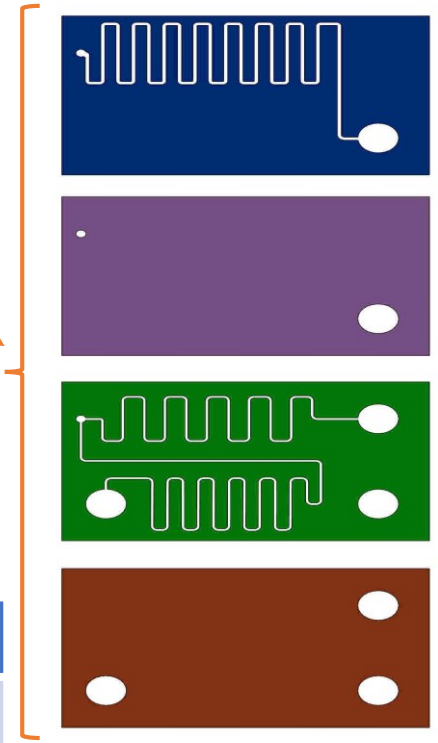
- Found fluid leakage through the lateral interface

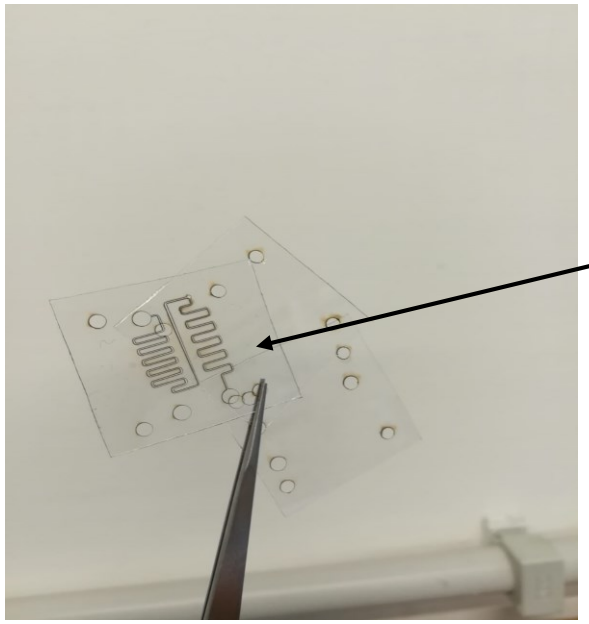
Fs Laser cutting of thin polycarbonate(PC) sheet

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Multilayer chip

- Layer-by-layer manufacturing technology
- Microfluidic system constructively divided into individual layers
- Microstructures on each layer are formed separately by laser cut
- All layers are stack together and joined to form a single chip





PC

Samples cut by using Femtosecond laser

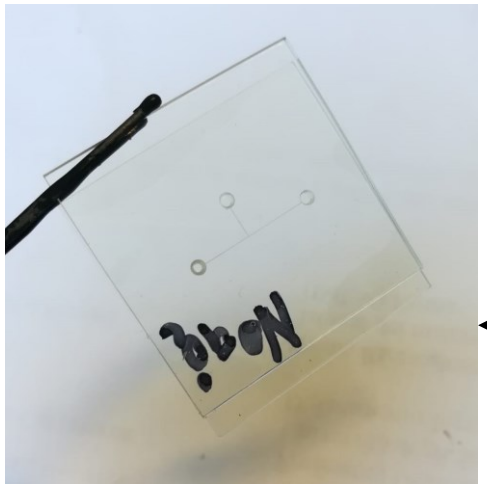
Laser parameters	
Frequency	50KHz
Power	0.4W
Laser scan speed	40mm/s
Short pulse energy	8.1µJ
Number of loops	10
Hatch distance	5µm

Bonding of microfluidic devices

- Bonding is the most important and final step of microfabrication
- Conventional methods using: thermal fusion bonding, chemical bonding and solvent bonding

1. PMMA- PMMA bonding

- A solvent bonding using isopropanol(IPA)
- Advantages: cheap, deformation free, simple



Processing steps

Cleaning the sample materials with IPA

↓

Dry out the cleaned samples at room temperature(25˚)

↓

Pour few drops of IPA to the patterned surface of PMMA and cover it with plane PMMA slab

↓

Hitch up both PMMA slab together with a plastic clamp

↓

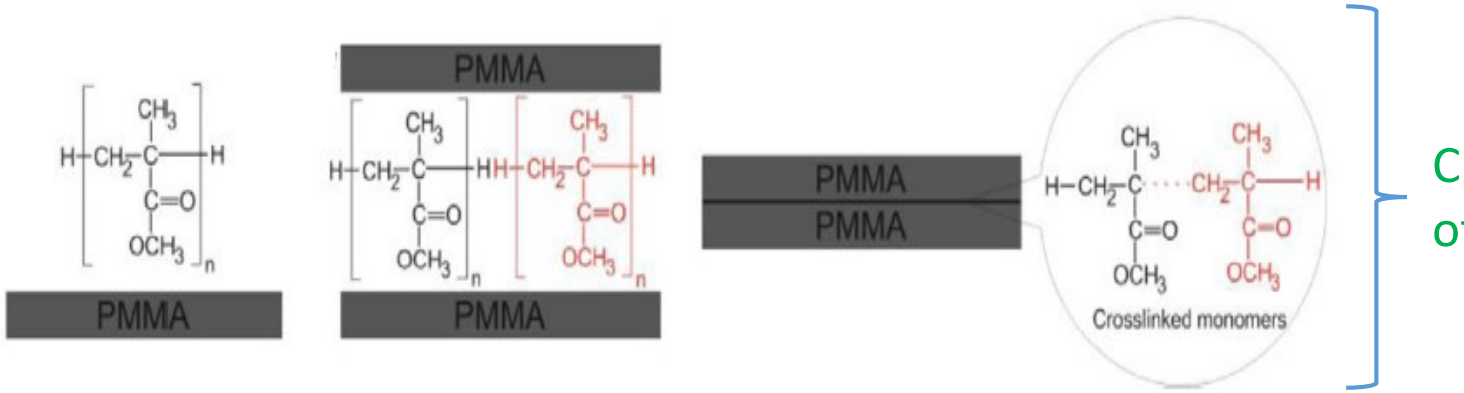
Put the sample into an oven for 5 minutes at 120˚C

↓

Sealed chip

Bonding of microfluidic devices

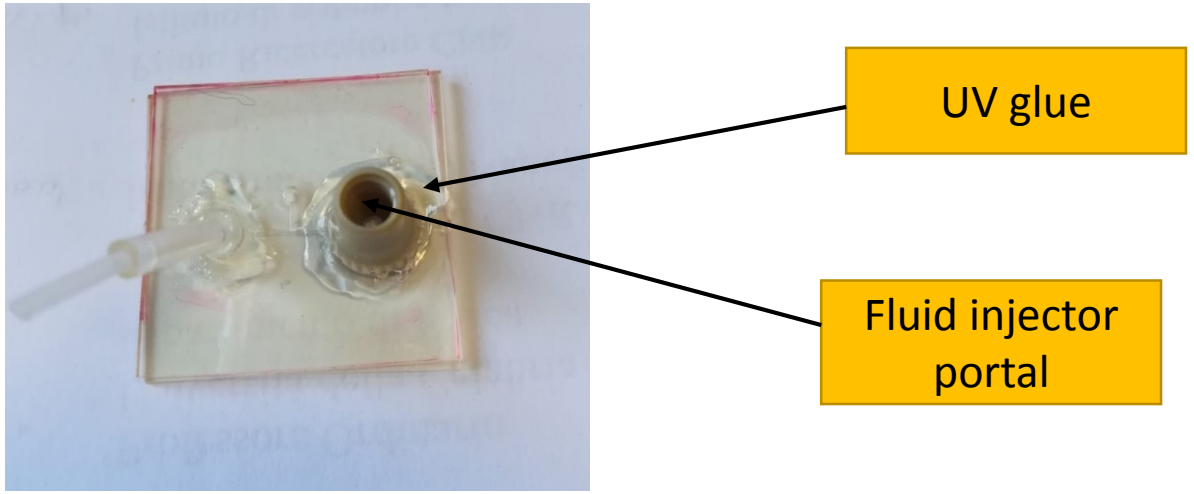
1. PMMA-PMMA bonding



Chemical reaction on the surface of the PMMA substrates

Testing of fluid flow through the microchannels

- Fixed a fluid injector portal at the inlet to inject the fluid inside



- Tested the fluid flow by pumping water into the microchannel by using a micropump

Bonding of microfluidic devices

2. PC-PC bonding

- One step solvent bonding
- Material used: polycarbonate(PC), acetone, pentane

Processing steps

Prepare a solution of the mixture acetone and pentane (3:7 ratio)

↓

Pour the solution to all the layers to be bonded

↓

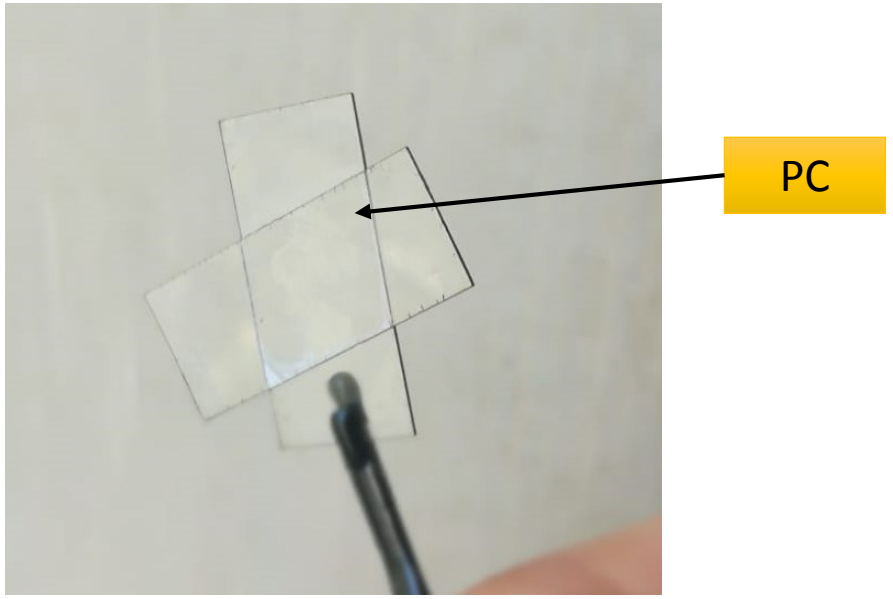
Hitch up all the layers together in the manner of final chip

↓

Put the samples on a hot plate for 15s at 60˚C

↓

Bond all layers together to make a single chip



Example for bonded PC layers

Future work

Integration of laser ablated PMMA microdevice into neuroscience research:

- Try to culture neuronal cells on a 5mm thickened laser ablated PMMA device
- Testing press fitting PDMS blocks enclosure system with CNC machined moulds

Lab on a chip for DNA extraction:

- Manufacture and assembling all the building blocks of the polymeric lab on chip that can extract DNA from biological samples
- Validation of final device

- **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

- **Conferences**

1) International symposium “Fundamentals of laser assisted micro and nanotechnologies at Saint Petersburg, June 30- July 4, 2019.

“Femtosecond laser micromachining of a polymeric Lab on a chip for particle sorting” – Oral presentation

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Thank you