Departimento Interateneo Di Fisica "M. Merlin"
Dottorato di Ricerca in Fisica XXXII ciclo

Femtosecond laser based smart procedures for the fabrication of polymeric lab on a chip devices

Industrial PhD with ST Microelectronics Lecce
(Tutor: Ing. Francesco Ferrara)

3 year Ph.D activity report

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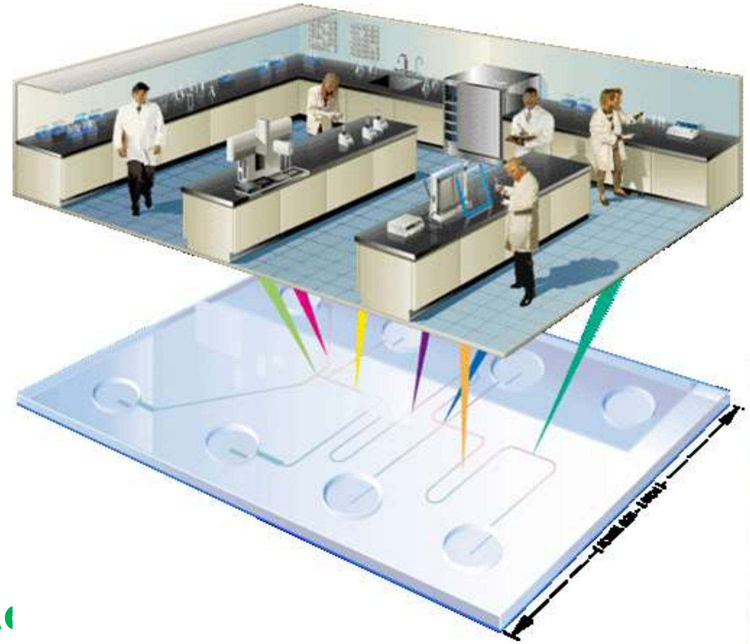
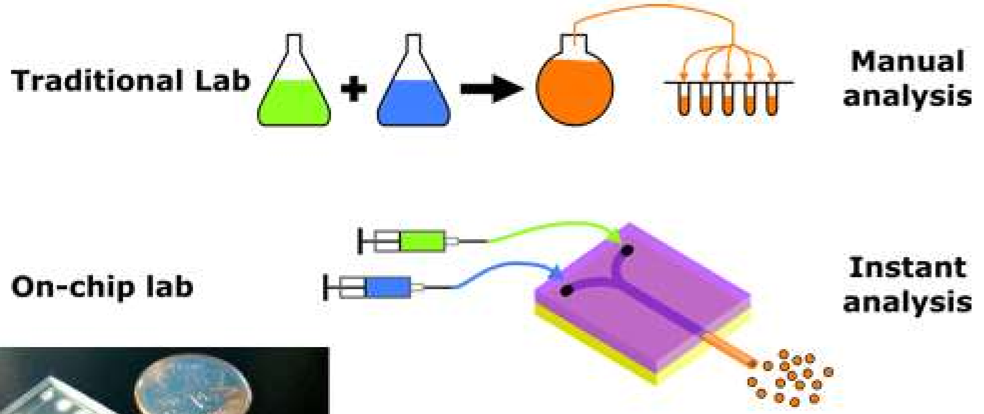
Outline

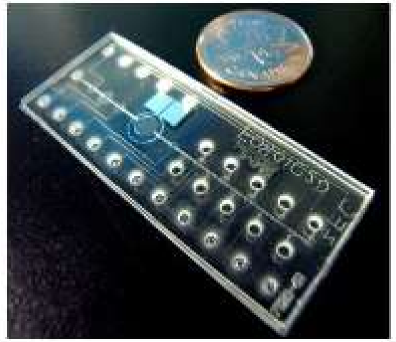
- Introduction
  - Lab-on-a-chip (LOC)
  - Materials used for LOC
  - Methods used for prototyping LOC
- Aim of the work: Smart procedure for prototyping polymeric LOC
- Experimental setup
- Design of Experiment (DoE) approach for optimizing laser parameters
- Results
  - Circulating tumour cell(CTC) capturing device fabrication
  - Bonding of CTC device
  - CTC capturing
  - Neuronal cell culture device fabrications
  - Neuronal cell culturing
- Conclusions

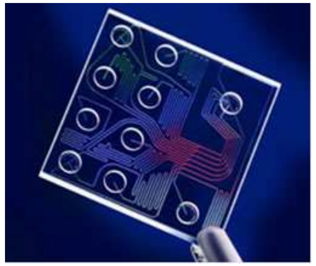
Lab-on-a-Chip (LOC)

Microfluidic devices integrating one or several laboratory functions on a single chip









A

- Low fluid volumes consumption
- Faster analysis and response
- Limited exposure to dangerous chemicals
- Reduced manufacturing costs
- Integration of functionalities
- Compactness
- Parallelization → high throughput analysis

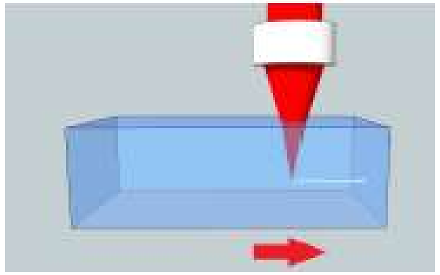
Disadvantages

- Novel technology not fully developed
- Processes more complex and detection difficult at the microscale
- **High precision required for their microfabrication**

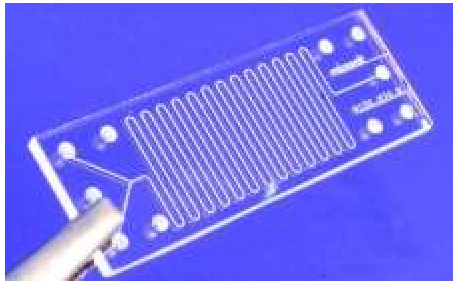
Materials used for LOC

- Silicon

- Glass



- Polymers



WHAT WE NEED

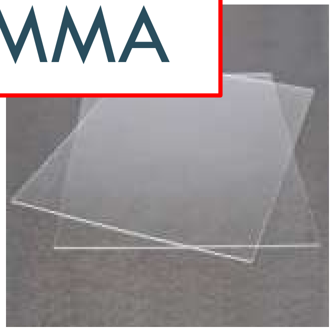
- Chemical stability
- Biocompatibility
- Optical transparency
- Mass production
- Excellent replication fidelity
- Low cost

Silicon (Chemical stability, Biocompatibility)

Glass (Chemical stability, Biocompatibility, Optical transparency)

Polymers (all of the above)

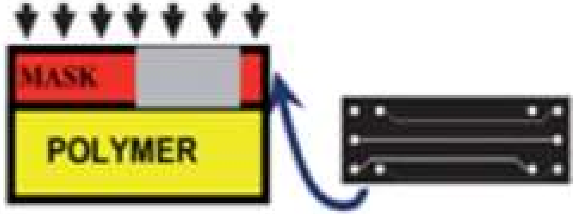
PMMA



- Rigidity
- Transparency in the visible spectral range
- Biocompatibilty
- Good environmental stability

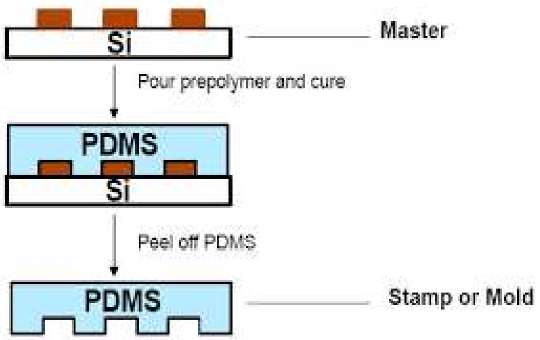
Methods used for prototyping LOC

1. Photolithography



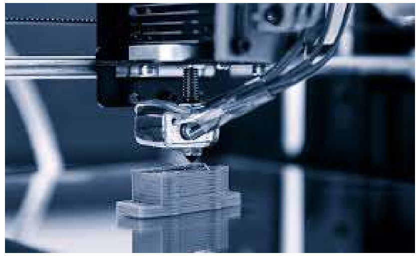
- **Flexible**
- Expensive
- Low production rate
- Need a mask

2. Soft lithography



- **Low feature size**
- **Low cost**
- Need an extra step for mould fabrication
- Soft material (PDMS)

3. 3D printing

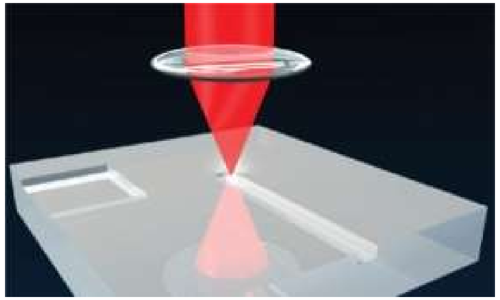


- **CAD file to device**
- Resolution
- Time consume
- Not transparent material

4. Mechanical micro milling

- **CAD file to device**
- **Material choice**
- **Large feature size in less time**
- Precision

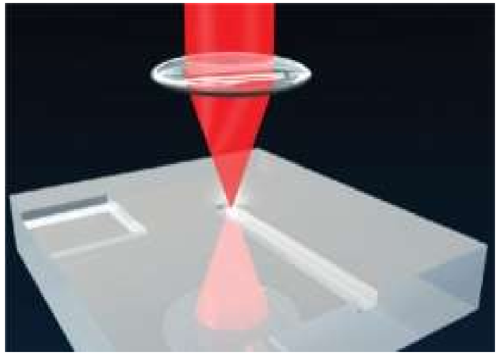
5. Fs laser microstructuring



- **Flexible and CAD file to device**
- **Precision and low feature size**
- Expensive
- Large area structuring
- Tapering

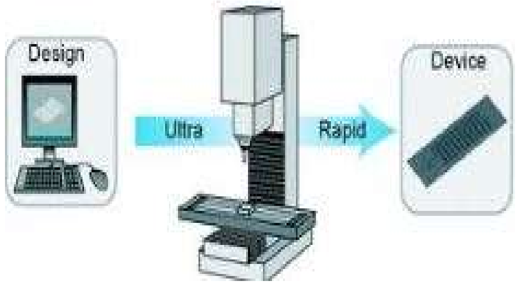
Smart procedure for prototyping polymeric LOC

Fs laser micro structuring



- **Flexible**
- **CAD file to device**
- **Precision and low feature size**

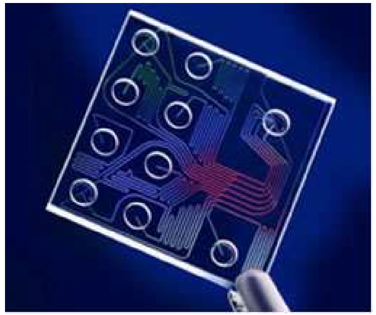
Mechanical micro milling



- **Large feature size in less time**

Solvent assisted thermal bonding

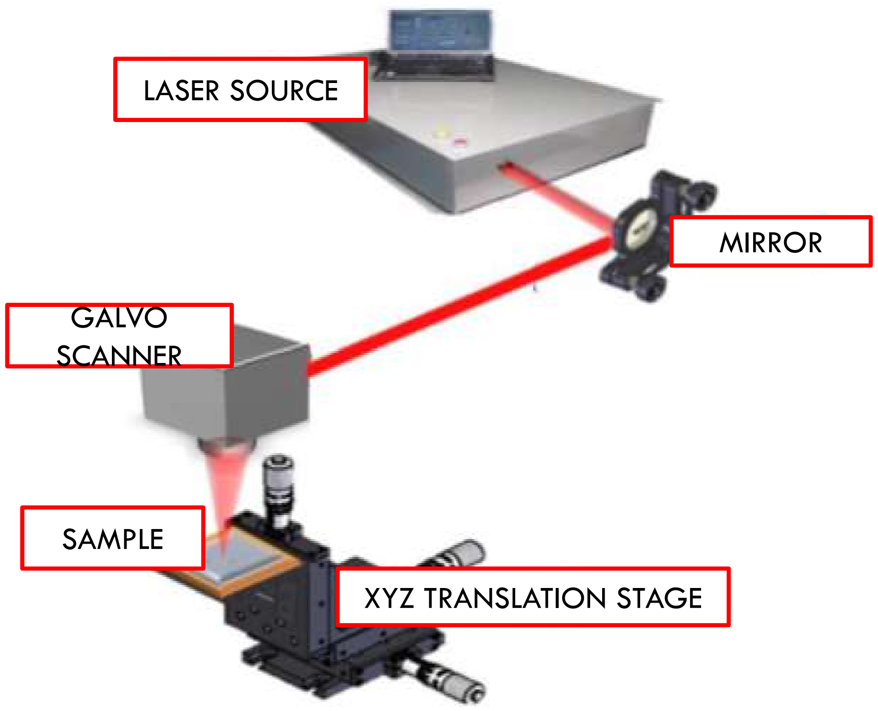
- **Cheap and simple**
- **fast**
- **Deformation free**



Complete device

Experimental setup

Fs laser system

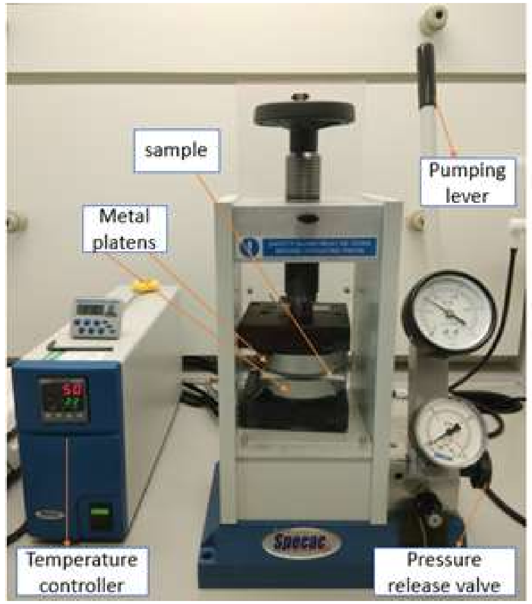


Laser system : TruMicro 5050 Femto Edition laser
Wavelength : 1030nm
Pulse duration : 900fs
Max. Power : 40W
Max. Pulse energy : 400µJ

Mechanical micro milling machine

Machine : Minitech CNC Mini-Mill/GX
XYZ axis travel: 300mm x 200mm x 200mm
Feed rates: 0.1 to 100 IPM linear; 0.1 to 50 IPM 3-axis simultaneous
Repeatability: +/- 0.002 mm
Spindle rotation: 90 degrees in both directions

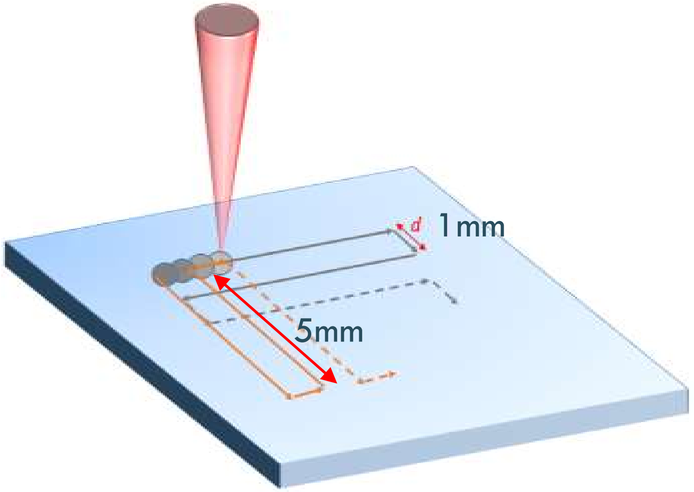
Hot embosser

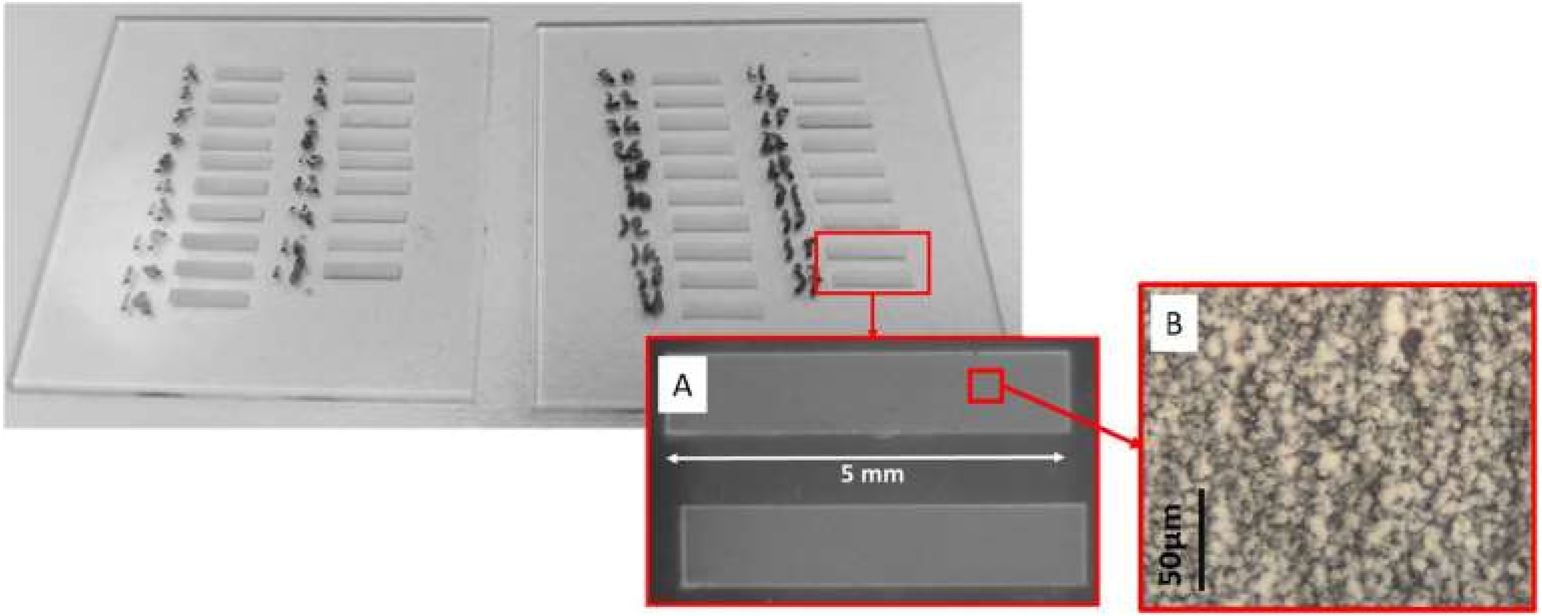


Machine : Specac Atlas manual hydraulic press 15T
Customized capacity: 0.9 T
Associated components: Temperature controller & chiller

DoE approach for optimizing laser parameters

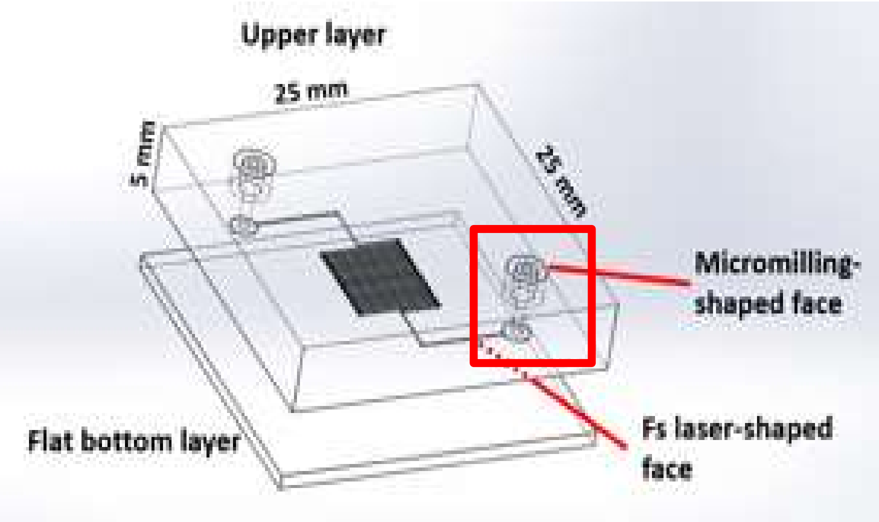
- Methodical way to quickly determine the laser process settings
- Predictive model for describing the relationship between variable depth and laser parameters
- Estimated the influence of laser R.R, pulse energy, scanning speed and hatch distance
- Test was performed super imposing two perpendicular scanning patterns
- Two level full factorial design with resolution V is defined





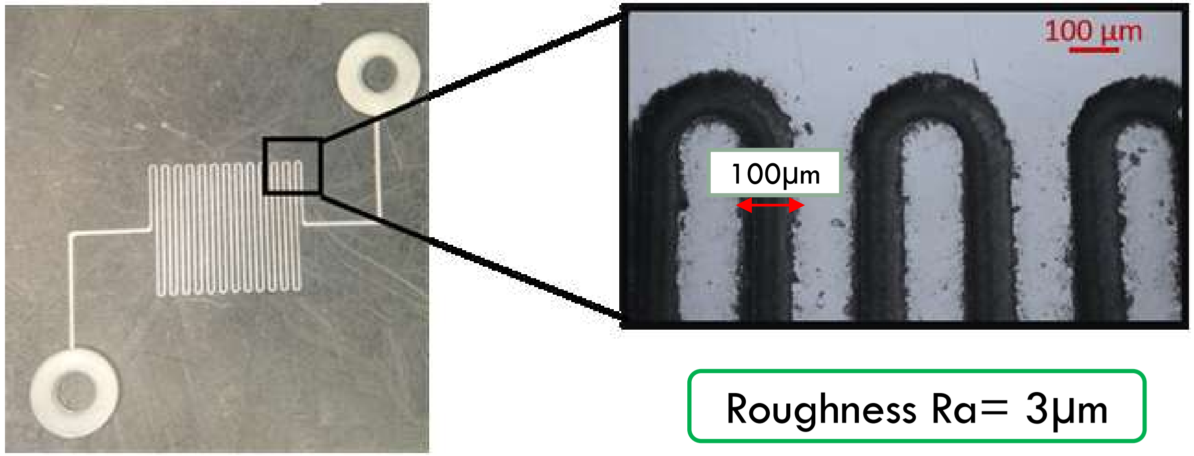
- Pulse energy and R.R are the main factors affecting the depth

Results: Circulating tumour cell(CTC) capturing device fabrication



Device design and fabrication

- Serpentine shape microchannel : total length=180mm
- Square cross section : 100µm x 100µm
- Increase active path and probability of capturing cells
- 2 PMMA substrates used: (1)micropatterned and (2)plane for sealing
- Serpentine microchannel on the lower face by Fs laser ablation
- Inlet/outlet holes drilled on the upper face by mechanical micro milling



Roughness Ra= 3µm

Laser parameters	
Repetition rate (R.R)	50KHz
Short pulse energy	12µJ
Scan speed	40mm/s
Hatch distance	5µm

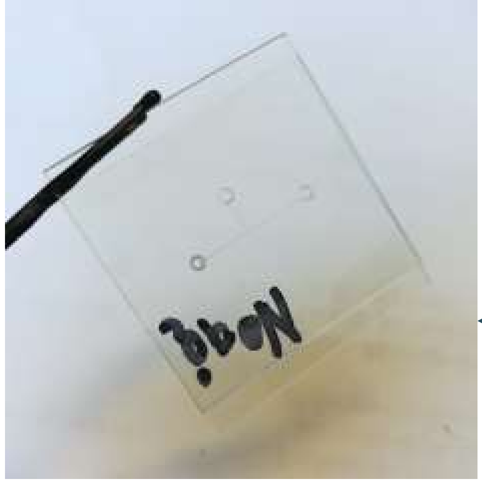
Result: Bonding of CTC device

- Isopropyl acid (IPA) assisted thermal bonding
- Advantages: cheap, simple, fast and deformation free

Tested the fluid flow without any leakage by pumping water into the microchannel using a micropump

Processing steps

1. Cleaning the PMMA substrates with IPA
2. Dry out the cleaned substrates
3. Spin coating(2000 RPM) of hot IPA (70°C) on plane PMMA
4. Stack the substrates together by a plastic clamp
5. Put the sample into a pre heated(60°C) oven for 20 minutes
6. Bonded chip

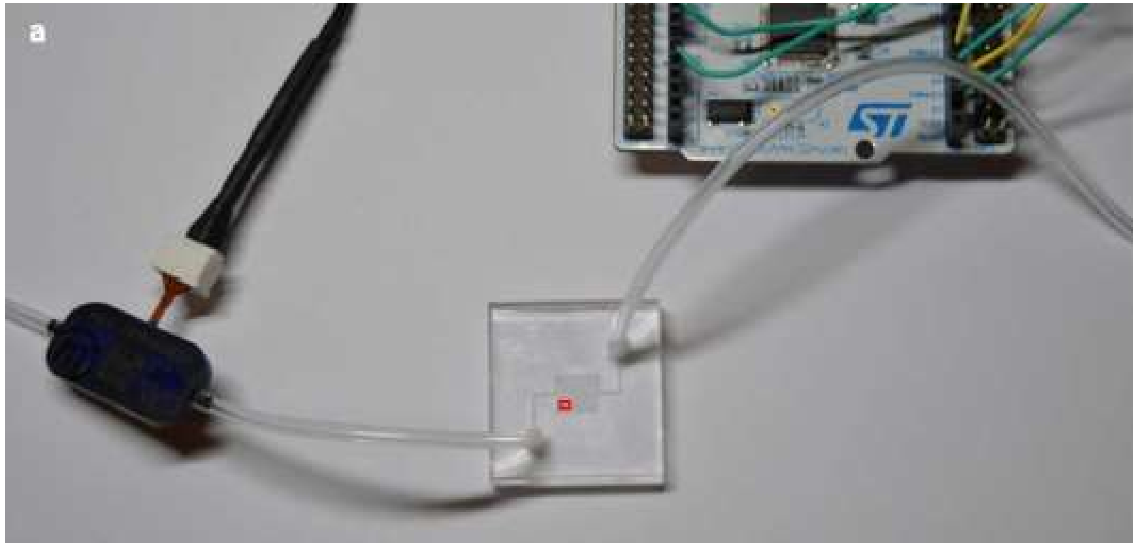


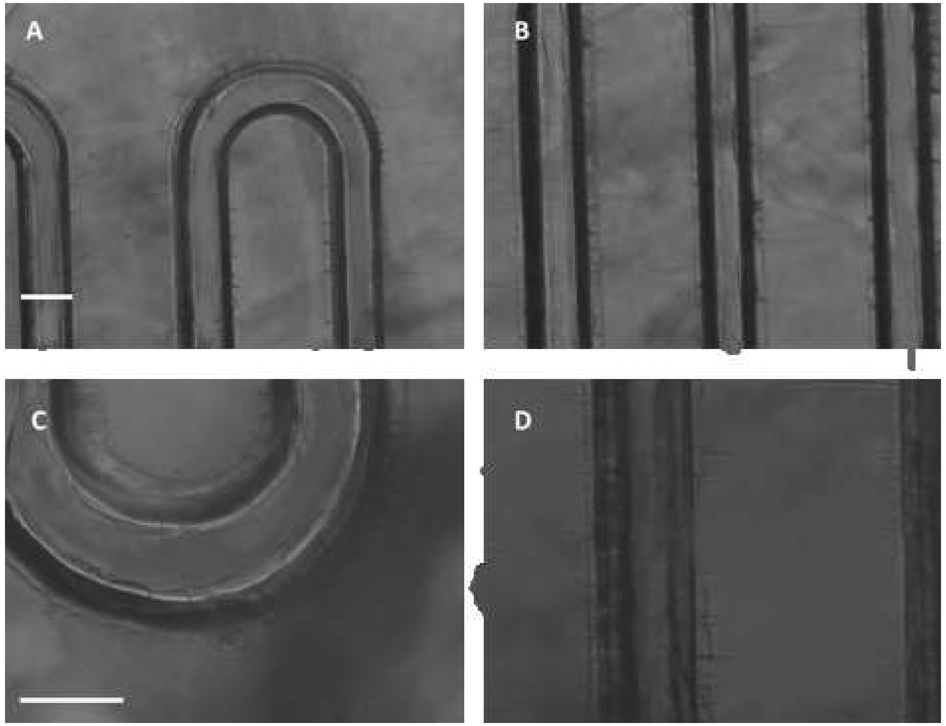
Result: CTC capturing

- The microchannels were functionalized with Anti EpCAM antibodies
- Tested the device to capture cancer cells from a mixture of normal and tumor cells
- Prepared 5ml suspensions of 10^6cells/ml Jurkat line cells and 10^4cells/ml OECM-1
- The cell suspensions were allowed to flow slowly through the serpentine channel with a flow rate of 8μl/min

Result for Jukat cells

- No or few Jurkat cells were identified in the channel after washing with PBS solution

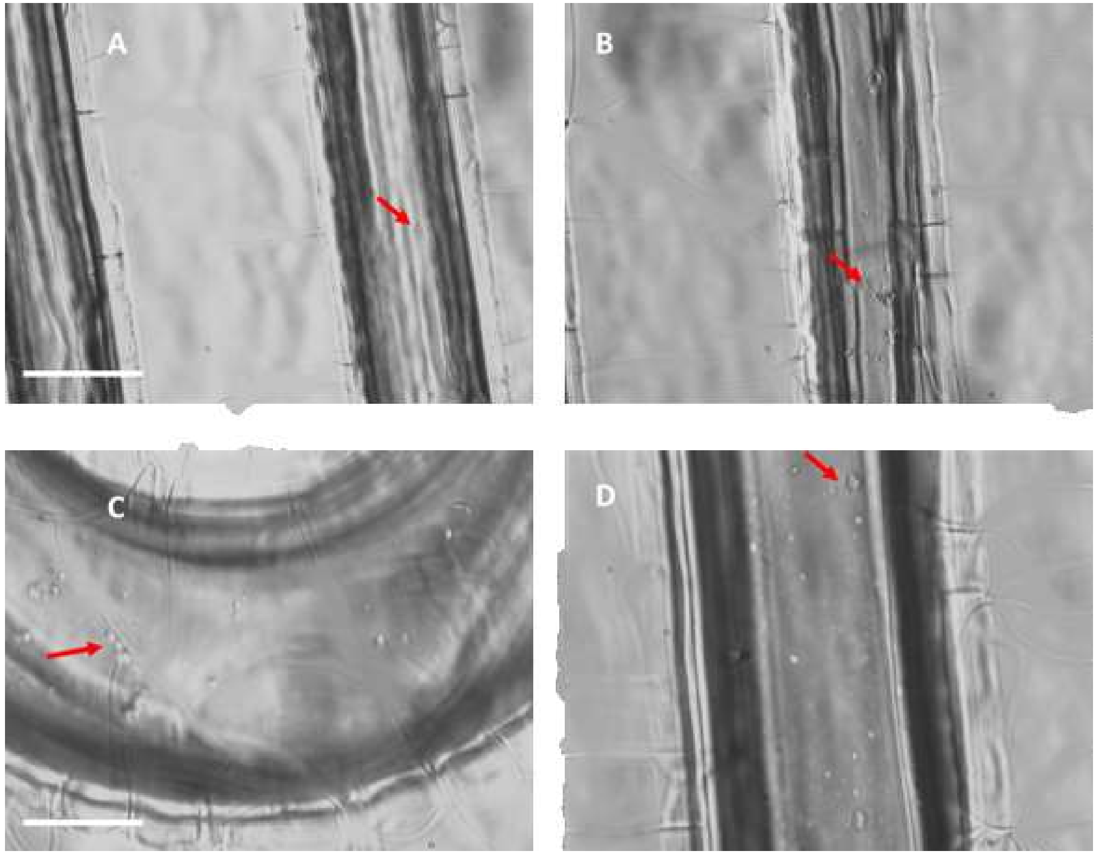




Result: CTC capturing

Result for OECM-1 cells

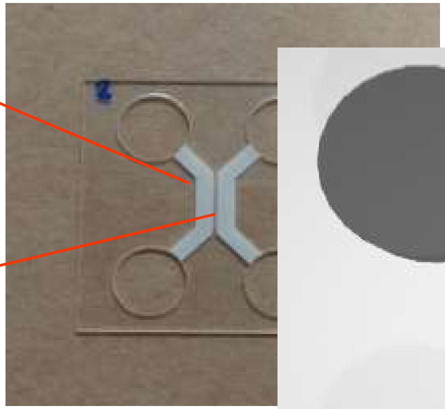
- High number of OECM-1 cells were captured on the inner surface of channel

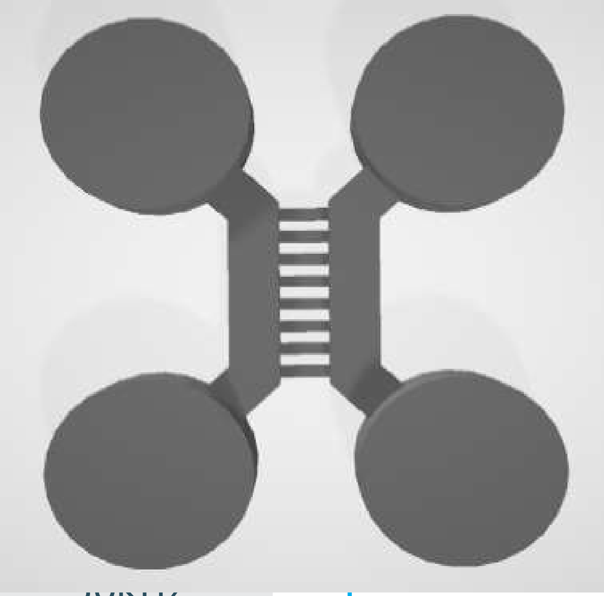


Result: Neuronal cell culture device fabrications

- Neuroscience investigates the basic function of nervous system
- Soft lithography on PDMS is conventionally used for the fabrication of devices
- Device design: 2 big channels and series of microchannels

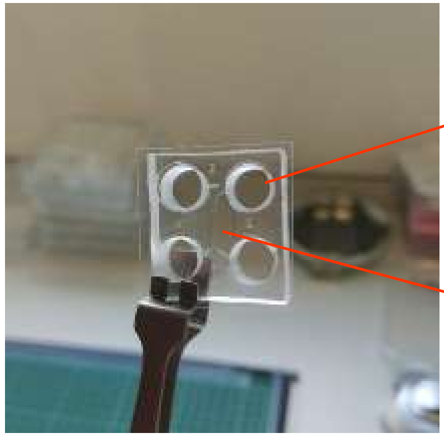
Laser ablated device





	Laser para	
	Microcahnnel array	
Frequency	0.625KHz	50KHz
Power	0.010W	0.6W
Laser scan speed	1mm/s	25mm/s

Hot embossed device

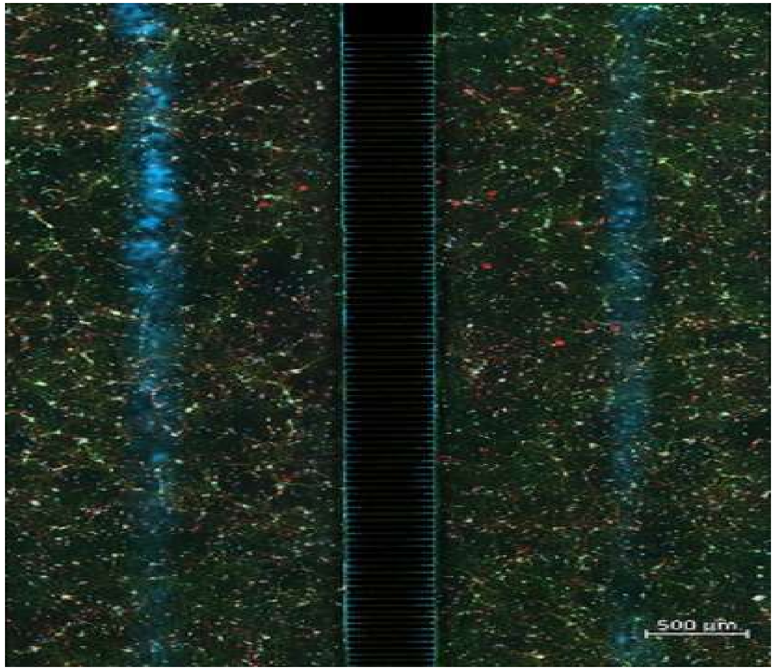


Loading temperature	**120°C**
Loading time	15 min
Cooling temperature	22°C
Cooling time	1 hour
Pressure load	0.2 ton

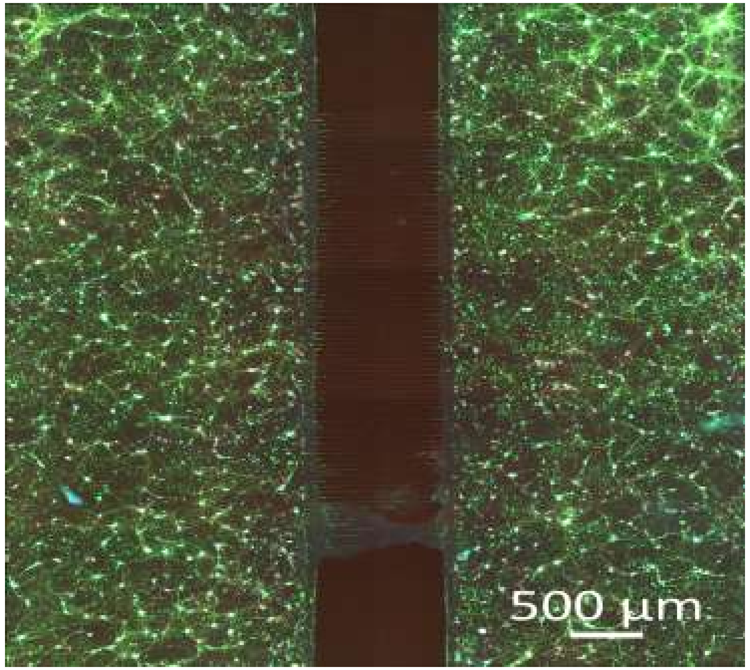
Result: Neuronal cell culturing

- Primary hippocampal cells were cultured in both devices
- Immunocytochemical staining is used for imaging of cell culture

Laser ablated device



Hot embossed device



- βIII-tubulin staining (green) reveals cellular networks formed between neurons
- This suggests PMMA does not hamper growth of primary neuronal cultures

Conclusions

- DoE procedure has been defined and optimized the laser parameters for microstructuring
- A microfluidic PMMA device has been realized and tested its functionality in capturing tumor cells
- To this purpose a smart and cost effective procedure has been established based on three steps:
    1. Fabrication of the microfluidic network was done by combining Fs-laser and mechanical micro milling
    2. The device has been assembled through a facile and low cost solvent assisted thermal bonding method
    3. In flow and on chip functionalization of the fabricated microchannel

- PMMA microfluidic devices have been fabricated by laser ablation and hot embossing
- Both devices were tested for culturing of neuronal cells
- The adoption of these fabrication procedures allows to easily prototype devices for many different applications

Ph.D activity

Università degli Studi di Bari (1.5 years)

- DoE Approach for Optimizing the laser parameters
- Fabrication of laser ablated CTC and neuronal cell culture devices

STMicroelectronics (1 year)

- Optimization of bonding technology
- Functionalization and validation of CTC device

University of Strathclyde (6 months)

- Fabrication of hot embossed device for neuronal cell culture
- Neuronal cell culturing and imaging

Course work

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	20	2	Final test
Introduction to parallel Computing and GPU Programming using CUDA	Pantaleo	June	16	2	Final test
Fluidodinamica computazionale	Pascazio Giuseppe	September-October	40	4	Final test
Optical sensors and spectroscopic tecniques	Spagnolo/ Patimisco	June-July	20	2	Final test
Total				16	

Publications

- "Prediction model of the depth of the femtosecond laser micro-milling of PMMA", Annalisa Volpe, Gianluca Trotta, Udith Krishnan, Antonio Ancona. Optics and Laser Technology 120 (2019) 105713. https://doi.org/10.1016/j.optlastec.2019.105713
- "Smart procedure for the femtosecond laser-based fabrication of polymeric lab on a chip for tumor cells capturing", Annalisa Volpe, Udith Krishnan, Maria Serena Chiriaco, Elisabetta Primiceri, Giuseppe Maruccio, Antonio Ancona, Francesco Ferrara – Submitted in Engineering journal

Poster presentations

- "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

Conferences

- 21st International Symposium on Laser Precision Microfabrication, 23-26 June 2020 – Dresden, Germany, "Femtosecond laser based smart procedures for the fabrication of polymeric lab on a chip devices"- Oral presentation

Summer schools

- 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" – Talk

Thank you

Acknowledgement :