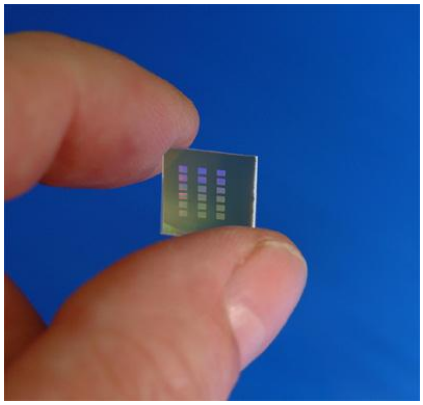
2_ PUBLISHABLE SUMMARY

microFLUID (micro-Fabrication of polymeric Lab-on-a-chip by Ultrafast lasers with Integrated optical Detection) is an FP7 STREP project within the ICT Priority. It is coordinated by Dr. Roberto Osellame, Institute of Photonics and Nanotechnologies (IFN), National Research Council (CNR), Italy (e-mail: roberto.osellame@ifn.cnr.it) and involves the following contractors:

- Istituto di Fotonica e Nanotecnologie- CNR (Italy)
- Laser Zentrum Hannover (Germany)
- Institut d'Alembert - ENS Cachan- CNRS (France)
- Politecnico di Milano (Italy)
- University of Manchester (U.K.)
- Agrolabo SpA (Italy)
- Centre Suisse d'Electronique et de Microtechnique (Switzerland)
- Microfluidic Chip Shop GmbH (Germany)
- Institut für Mikrotechnik Mainz GmbH (Germany)

The project website is: http://www.ifn.cnr.it/microfluid/.



Lab-on-chips (LOCs) are microsystems capable of manipulating small (micro to nanoliters) amounts of fluids in microfluidic channels with dimensions of tens to hundreds of micrometers: they have a huge application potential in many diverse fields of chemistry and life sciences. Notwithstanding their potential, LOC commercial exploitation has been slow so far. Two breakthroughs that could promote LOC diffusion are: (i) a microfabrication technology with low-cost rapid prototyping capabilities; (ii) an integrated on-chip optical detection.

Polymers are rapidly emerging as the material of choice for the LOC substrates, due to their low cost, favourable optical properties and ease of processing. Many of them are already commercially available, but an almost unlimited number of modifications in the composition are feasible for a further optimization of the chosen structure.

Laser fabrication is a flexible, low-cost and high-throughput process for the creation of a variety of microstructures in a broad range of materials. In particular **femtosecond lasers**, due to their peculiar light-matter interaction regime, offer unique microfabrication capabilities. For a long time their use has been practically limited by their high cost and their complexity. Recent technological advances have dramatically improved their performance and reliability, making them available as a practical micromachining tool. Femtosecond laser fabrication can be employed in different steps of the LOC production cycle: (i) the rapid prototyping of the microfluidic chip; (ii) the direct fabrication of photonic components on the LOC for integrated optical sensing (iii) the master fabrication for mass production by replication techniques.

The main objective of the microFLUID project is **the use of femtosecond lasers as a novel highly flexible microfabrication platform for polymeric LOCs with integrated optical detection**, for the realization of **low-cost and truly portable biophotonic microsystems**.

The scientific developments of the project will be applied to two **prototypical devices**:

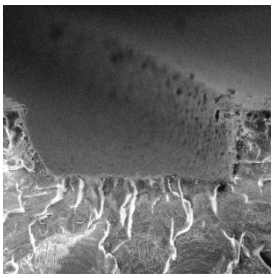
LOC for the detection of mycotoxins in animal feeds: **Mycotoxins** are toxic metabolites produced by fungi **growing on foodstuffs or animal feeds**. Mycotoxins enter the human food chain via meat or other animal products as the result of livestock eating contaminated feed. It is now well established that diseases caused by mycotoxins have been responsible for major epidemics in man and animals. For this reason it is of great importance to develop **sensitive, accurate and cost-effective tests** for the detection and quantisation of mycotoxin in feeds.

Water screening in LOCs for bacteria and heavy metal ions detection: **Water screening** is very important for improving **human health** and preserving the **environment**. In the case of **bacteria analyses**, quantitative bacterial culture is very time consuming and expensive. Therefore, it is very important to develop a reliable screening method for differentiation of liquid samples which contain significant numbers of bacteria from those which do not. In this project, **high-throughput flow cytometry** will be employed as a rapid and feasible approach for the detection of bacteria by size and shape sensitive light scattering methods.

Hg^{2+}, Cd^{2+} and Pb^{2+} are the most **toxic heavy metal ions**, causing adverse environmental and health problems. For these reason, the level of heavy metal ions in drinking water is the object of strict regulations. While sophisticated analytical techniques are currently used in applications relevant to massive environmental contamination, there is still a significant need to provide **inexpensive and real-time monitoring** methods for the detection of trace amounts of mercury and lead in **drinking water**, in **polluted areas** and in **industrial waste streams**.

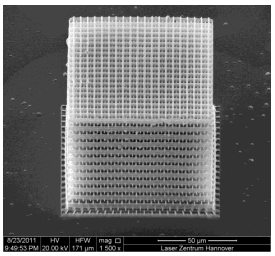
During the three years of the project, all the partners worked synergically towards the objectives and reached most of the project goals. The main achievements have been:

- **Femtosecond laser ablation of microfluidic channels** on several polymer substrates and identification of the best processing window combining surface quality with processing speed

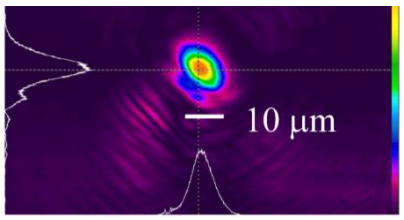


- **Morphological and chemico-physical characterization** of laser ablated surfaces to explain the observed permanent enhancement of the hydrophobicity. Development of a **chemical treatment to remove surface roughness and restore hydrophilicity**.

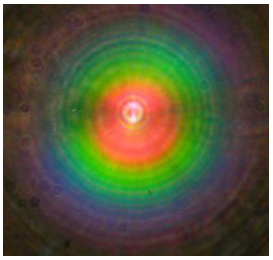
- **Development of new resins** (acrylic- or PFPE-based) for high quality **two-photon polymerization** and identification of the best processing window.



- **Optical waveguide writing** by femtosecond lasers in several polymer substrates, including PMMA and COP. Parameters for permanent waveguide fabrication have been identified. Guiding is demonstrated in the whole visible range

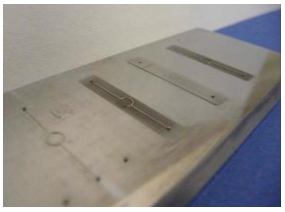


- **Fresnel lenses has been fabricated in polymeric LOCs to implement on chip optical sensing**: extremely precise surface laser ablation has been employed to integrate Fresnel lenses, with very short focal length, into microfluidic chips

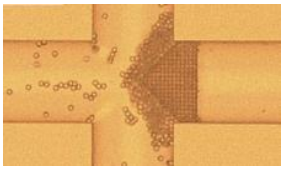


- **Development of LOC devices for the two applications of the project**: mycotoxin detection and water screening. The devices are designed from a system point of view including fluidic and optical functionalities.

- **Demonstration of a module for the extraction of mycotoxins from solid foodstuff**: an external module that can be directly interfaced with the LOC has been fabricated and characterized. It is capable of grinding solids and extracting their mycotoxin content.

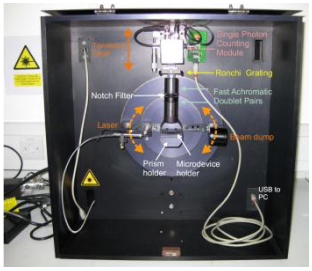


- **Steel master tools** have been fabricated by a new technology based on **mask-assisted femtosecond laser ablation**. High removal rate combined with high precision have been demonstrated.

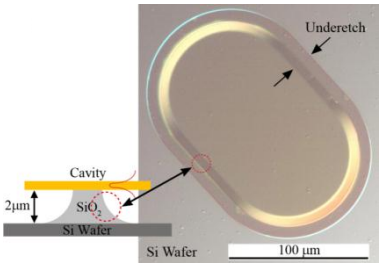


- Fabrication of **porous filters inside already sealed microfluidic channels by two-photon polymerization:** high-resolution, 3D capabilities and arbitrary geometry.

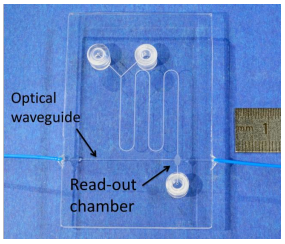
- **Development and validation of an immunoassay protocol to detect mycotoxins in a microfluidic chip**: correlation with highly sensitive HPLC measurements have a correlation >80% on all tested samples, extracted from contaminated foodstuff.



- **Development of a flow cytometer prototype for bacteria detection:** a flow cytometer for particle detection by multichannel correlation has been built and validated with 10 µm diameter beads.



- **Heavy metal ions detection by a functionalized PMMA pedestal stadium cavity :** ligand molecules specific for Pb, Cd and Hg have been grafted on a doped PMMA microcavity. Laser spectrum shifts are a sensitive monitor of the presence of ions in a flowing water sample.



- **A microfluidic chip, fabricated by femtosecond laser ablation, can detect heavy metal ion based on fluorescence sensing**: A Y-channel with micromixing elements will be used to perform a complexation reaction between the fluorescent probe and the heavy metal ion. Monitoring the fluorescence signal with integrated optical fibers, ion concentrations as low as 1 µg/L can be detected.

In summary, microFLUID project on the one hand generated a large amount of basic multidisciplinary knowledge; while on the other hand a considerable work has been performed towards real-world applications with the development of several prototypes for mycotoxins detection and water screening.