
Doctoral Dissertation
Doctoral Program in Electrical, Electronics and Communications Engineering
(33th Cycle)

# Microfluidic devices: application for liquid biopsy

**Federica Barbaresco**
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**Supervisors**
Prof. C. F. Pirri, Supervisor
Prof. M. Cocuzza, Co-Supervisor

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# Summary

Lung cancer is nowadays a leading cause of death, second only to cardiovascular diseases. Thus, the large diffusion and incidence make the development of new diagnostic tools of crucial importance for lung cancer early detection, prognosis and treatment. In recent years, due to research improvements in cancer studies and biomedical technologies, liquid biopsy, a non-invasive and real-time monitoring approach, resulted a promising tool for the early diagnosis of cancer alterations. Moreover, this highly sensitive technique permits to control the evolution of lung cancer at different stages contributing to adjust therapy according to a personalized patient's treatment. Liquid biopsy concerns the analysis of biomarkers (i.e. cells, extracellular vescicles, sequence of nucleic acids, etc.) from blood or any other body fluids into integrated and automated microfluidic systems for point-of-care or in-the-field detection. Thus, the development of enhanced microfluidic devices, thanks to their characteristics, could overcome some critical aspects derived from traditional approaches, leading to a widespread application of liquid biopsy in everyday use either in diagnostic and in clinical practice.

In this contest, this PhD Thesis focuses on the development of three microfluidic devices for the separation or detection of biomarkers for the early diagnosis and screening of lung cancer treatment. In detail, each microfluidic device is referred to a specific class of biomarker. Thus, for each chip a proper design and fabrication method were selected depending on its functionality. Then, devices feature dimensions and their performances were investigated either with synthetic and biological samples employing different approaches.

At first, a novel free-flow microfluidic device was developed exploiting the 3D printing technology to manipulate the motion of particles and exosomes. Morphological characterizations of device features dimensions highlighted how an accurate reproducibility of the design was performed employing an additive manufacturing method. Moreover, 3D printing allowed to easily integrate threated fluidic fittings features in the chip in correspondence to inlet and outlet ports for tube interconnection by modifying a preliminary design of the device. Results performed with this device demonstrated their ability to tune the motion of analytes, owing different surface-to-charge ratios (i.e. particles and exosomes) either as single or mixed population, in a specific outlet when a defined voltage value at the electrodes was imposed and to accumulate them in a microliter volume range.

A second microfluidic device was developed throughout a silicon micromachining fabrication process to separate biomarkers possessing micrometric dimensions by exploiting acoustic forces. To allow the creation of an acoustic standing wave field into the microfluidic channel a customized set-up, with related protocol steps, was assembled and investigated. Afterwards, device focalization performances were evaluated by inspecting samples composed either by polystyrene micro- and nanoparticles and cells as single or mixed populations collected at the outlets of the device when different setting conditions (i.e. sample concentration, applied voltage value at the piezoelectric element and flow rate) were applied. Regarding micro particles tests, higher focalization performances were proven when lower concentration, lower flow rate and higher applied voltage value to the transducer were imposed. In addition, confirmation of these data resulted by comparing them to simulated ones. Furthermore, high focalization values with cells resulted both at high flow rate and when cells were mixed with population of micro- and nanoparticles without affecting focalization performances of the device.

Finally, two PDMS-based microfluidic devices, characterized with different surface-to-volume ratios, were designed and evaluated to capture low amount of microRNA molecules. Results performed throughout PCR reaction assays either with synthetic microRNAs and a pool of microRNA molecules associated to non-small cell lung carcinoma spiked in water or human plasma demonstrated higher performances of these devices to capture biomarkers with respect to a previous PDMS-based device, due to their higher availability of internal surfaces. Moreover, a rapid and automatized set-up for the capture of microRNAs was assembled and presented.

To conclude, microfluidic devices developed during this PhD Thesis work constitute proof-of-concepts for the development of new diagnostic tools for liquid biopsy. Further improvements regarding the presented chips, as well as their integration into a unique microfluidic platform will offer a new, rapid, low cost and multiple-analysis diagnostic tool able to overcome current challenges.