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On-Chip high-throughput LSFM analysis of single cancer cells

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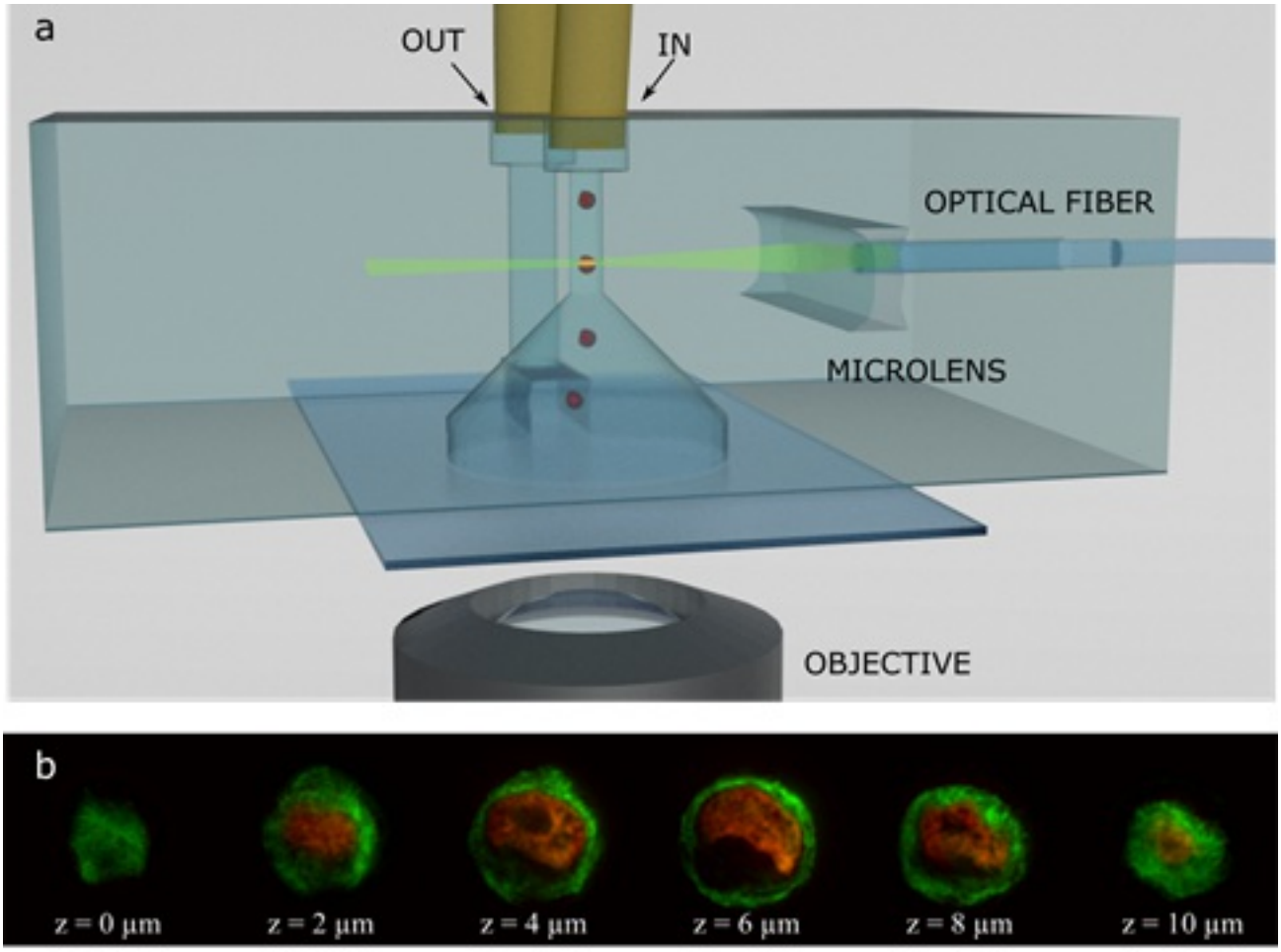
Abstract Text

The study of cellular populations heterogeneity is becoming a standard in biology, thus single-cell analysis techniques able to process tens or hundreds of cells per minute are required to collect statistically significant quantity of data in a reasonable amount of time. In this framework, fluorescence cytofluorimetry showed the ability to analyse large number of samples in a fast, automated and user-friendly fashion. However, the information that can be obtained from this technique are limited, like presence, absence or intensity of a specific fluorescence signal, or widefield 2D images. Light sheet fluorescence microscopy can play an important role in this field, by combining its fast imaging capability with fluidic continuous sample delivery.

We present an integrated all-glass device where an engineered microchannel is used to handle and deliver single cells, one after the other, in an automated fashion. The samples are flown in the channel through a prealigned light-sheet, allowing continuous acquisition. A cylindrical microlens is embedded in the chip, in order to shape the light-sheet directly on the same device, with no need of external optical systems, guaranteeing a robustness and precise alignment. The chip, with a footprint smaller than a coin, is designed to be easily mounted on a standard inverted microscope as a functional add-on. A scheme of the device is presented in Figure 1.a.



The whole device is realized using femtosecond laser micromachining, a versatile technique that allows the fabrication of 3D structures in biocompatible and inert materials like fused silica glass. The precision of the technique allowed us to shape the microchannel in order to reduce to the minimum the optical aberration in signal detection and to optimize the microlens profile, to be compatible with different excitation wavelengths.

We demonstrated the performances of our device by acquiring dual-color 3D stacks of hundreds of single cancer cells with a totally automated system, driven by a custom software. Each cell can be analysed in less than one second, still ensuring a three-dimensional resolution that allows the identification of cellular structures and sub-nuclear vesicles. In Figure 1.b a sequence of acquired sections of the same cell is reported. We believe that our system could be a powerful tool for cellular population studies and inspection of cancer cell heterogeneity and epigenetic alterations.