SINGLE-CELL BIOPHYSICAL AND KINEMATIC ANALYSIS BY MEANS OF LABEL-FREE TRACKING METHODS FOR DIAGNOSTIC APPLICATIONS IN MICROFLUIDICS

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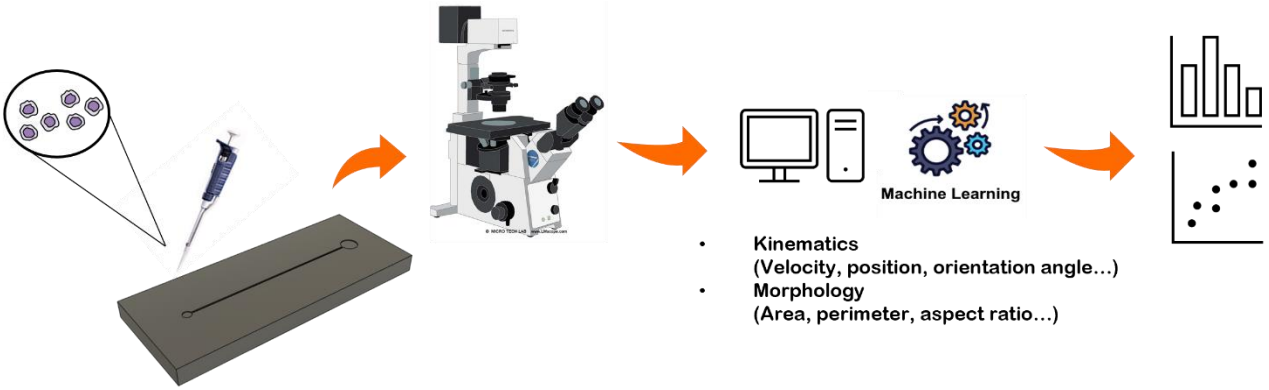
One major challenge in biomedical research is unravelling the complexity of cellular processes. Some of them, like intracellular responses to different molecules and signalling pathways, are still to be fully discovered. As a matter of fact, the highly heterogenic nature of cells, even among the same population, creates more difficulties when studying cellular physiological responses to molecules in bulk analysis. In this context, single-cell analysis has developed as a novel technique for studying cellular interactions among other cells and the microenvironment under defined conditions. Compared to the average analysis of millions of cells together, single-cell technologies provide information about each cell, identifying different characteristics and cellular dynamics in a cell population. This is particularly useful for tumour progression characterization, genomics and gene profiling, proteomics, tissue regeneration, immune response, embryonic development [1][2]. Throughout the years, many different methods have been explored for single-cell biophysical properties' characterization. Mechanical properties for example have been evaluated through atomic force microscopy, optical tweezers and micropipette aspiration; electrical ones through patch clamp and nanoprobe technologies, optical properties through the measurement of refractive index. Some of the major drawbacks for these techniques are the difficulties in sample preparation, low throughput and the requirement for complex machinery [4].

Microfluidics (i.e., fluid dynamics manipulation in manufactured microminiaturized devices) is a promising technical platform for single-cell analysis; as a matter of fact, thanks to the miniaturization of processes, small amounts of sample can be elaborated with high precision detection, and quantitative analysis at cellular level can be performed. Moreover, high throughput capability and low cost of production are two more valuable assets of this technique [3]. Some microfluidic applications for label-free single-cell analysis include fluid-induced deformation for mechanical properties [6], flow cytometry for electrical detection of cells, and application of cell refractive index as regards optical properties [4].

Besides biophysical properties, kinematic analysis of cellular motion is also a valuable field of research for the understanding of cellular behaviour. Object tracking, which is the process of locating and following a specific object and its behaviour in sequential images, is a difficult task in biology because of the large size of cell image sequences, which makes the analysis more convenient to automatize. Computational object tracking in fact can highly improve reproducibility and computational time, although the very small cellular sizes often require the use of fluorescent labels to ease the location and tracking process, reducing the sample viability [5].
As already mentioned, single-cell analysis allows the gathering of different cells characteristics among a bulk, and thus has broad application in diagnostics. For example, the identification and separation of abnormal cells based on their biophysical characteristics is a useful possibility for individualization of therapy-resistant tumour cells or for identification of new biomarkers for cancer early diagnosis [7][8][9], identification of parasites in blood [10], male fertility evaluation [11] [12], characterization of immune cells ([13]) and neuronal cells [14].

This project aims at the development of a novel concept of single-cell label-free analysis on a microfluidic platform for diagnostics. Biological samples will be analysed in order to find key parameters for cell characterization. During the first year, we elaborated a single-cell analysis based on cell tracking in brightfield through the development of a MATLAB routine. Semen fluid was loaded in a microfluidic device and videos were acquired in a brightfield mode with an inverted microscope, equipped with a normal and a high-speed camera for recording image and videos. The automated routine that we are writing can extract kinematic parameters, related to the motion and, biophysical ones, related to morphology. This analysis does not require the use of staining, preserving the sample viability and making the process

fast and easily reproducible in a clinical context. A further goal is to feed the output of the routine to a machine learning algorithm to identify subcellular classes based on the extracted parameters.



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