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FluoLab: A New Easy-to-use Graphical User Interface for the Multi-cell Functional Calcium Signals Analysis

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Abstract – **The progress in fluorescence microscopy and information technologies have completely transformed the study on living cells improving the capability to quantify, investigate and analyze, in time and space, single phenomena occurring inside and outside cells. We developed an user-friendly Graphical User Interface (GUI) able to extract and analyze ion calcium (Ca^{2+}) signals and to understand how they regulate cell behavior and metabolism. The software, named *FluoLab* (Fluorescence Laboratory), works on acquired confocal fluorescence microscopy images and allows to obtain signals of Mean Fluorescence Intensity (MFI). Afterwards, the fluorescence signals are automatically converted in ion calcium concentration values, [Ca^{2+}], expressed in micro-Molarity (µM), by using the specific dissociation constant K_d for each kind of fluorescent probes chosen for the experiments. It is possible to analyze contemporaneously more than one object through the Region Of Interest (ROI) defined around them and to follow them in time. *FluoLab* can also show a normalization of the obtained data, compensating automatically the image background and generating a file that can be used for a fast data analysis.**

Keywords – biomedical measurements, bioengineering, signals and image processing

I. INTRODUCTION

Fluorescence microscopy, as experimental approach to the direct observation of biological processes in living cells, has received a powerful impulse in recent years thanks to different factors as technological progress of survey instruments and development of specific probes to mark and follow cellular structures or physiological parameters in *real-time* dynamic experiments [1]. In particular, with the development of confocal microscopy, image analysis systems have become increasingly complex: the mathematical analysis of traditional fluorescence images (digital imaging) allows the development of algorithms that calculate the contribution of out-of-focus portions of a biological sample. The other important element that allows to fully exploit the new technologies of image analysis is the availability of probes able to mark both living cell and subcellular elements (organelles, proteins, nuclei) or to measure important cellular parameters, such as calcium ion concentration [Ca^{2+}] [2, 3], an intracellular messenger, crucial for many biological processes [4, 5]. Calcium ions enter into the cytoplasm from intracellular stores or from the extracellular space, in this case driven by a large electrochemical gradient across the plasma membrane. That is the reason why calcium plays a crucial role in many differ cellular process and its pathway is a key point for cell life. Calcium is involved in excitability, exocytosis, motility, apoptosis, cell-cell interactions and gene transcription. Unfortunately, variety in cell types studied, different biological mechanisms investigated and different experimental conditions have led to the development of many analytical systems often associated with the instruments used for the acquisition of individual images (and thus of companies owned) or to the payment of a license to use software [6-10].

A topic of our laboratory is the study of the dynamic mechanisms that regulate calcium signaling, using different types of fluorescent probes in different cell models (megakaryocytes, platelets, mesenchymal cells, exosomes) [11-13]. For example we noticed that

megakaryocytes, in particular experimental conditions, show calcium mobilization from intracellular stores. This phenomenon represents the basis of a signal cascades activation that causes megakaryocyte adhesion, starts pro-platelet formation and promotes extracellular calcium entry, which is largely involved in the regulation of the contractile force related to megakaryocyte motility [12]. Megakaryocytes products are calcium dependent too: in platelets, calcium is a crucial agent for adhesion, activation and thrombus formation. Calcium agonists also enhance the generation of vasodilator and platelet anti-aggregant factors which could disempower platelet functionality [14]. Calcium plays an important role also in cell differentiation. Bone marrow-derived mesenchymal stem cells (MSCs) can be differentiated into myocytes, as well as adipocytes, chondrocytes and osteocytes in culture. The capability of a cell to receive and transmit a signal of calcium represents a key factor for a cell to become contractile. This is guaranteed by the expression of calcium channels that mediate the excitation-contraction coupling and are essential for the muscle functionality [15].

In order to optimize the analysis of images obtained by confocal and fluorescence microscopy, we developed a new easy-to-use software able to obtain quantitative and qualitative data expressed, for example, as concentration of calcium ion [Ca^{2+}].

II. RELATED RESULTS IN THE LITERATURE

The significant developments in research, through the use of very specific fluorescent probes and new detailed and precise acquisition systems, have greatly contributed to significant advancement in studying biological systems *in vitro*. Commercially, there are many image analysis systems using specific software for different cell types, as described for example in [6-10]. Some of them analyze the calcium signal as a global phenomenon quantifying and monitoring its temporal variations and providing global and individual calcium fluorescence intensity profiles [6]. Others focus on segmentation and time series extraction through specific control algorithms [7-9]. Finally there is software able to analyze single-cell signal in large cell populations measuring their fluorescence intensity over time [10].

Our software differs from them and shows more versatility, more visual immediacy in results and more quickness in obtaining signals.

III. DESCRIPTION OF THE METHOD

We developed a versatile user-friendly GUI (Fig. 1) able to extract different metabolic signals from dissimilar cell types (i.e. platelets, megakaryocytes, mesenchymal cells). Software utilization starts from acquired fluorescence images, with the possibility to load images of different dimensions and formats. *FluoLab* leaves full freedom to the user, of biological or medical but not technical extraction, to choose and define the specific local or global Region Of Interest (ROI) (i.e. groups of cells, a whole cell, nuclei, mitochondria, granules, exosomes) from which it is possible to obtain automatically a signal related to that region. The software is able to analyze contemporaneously 20 different objects for each image and to follow the region signals in time, compensating

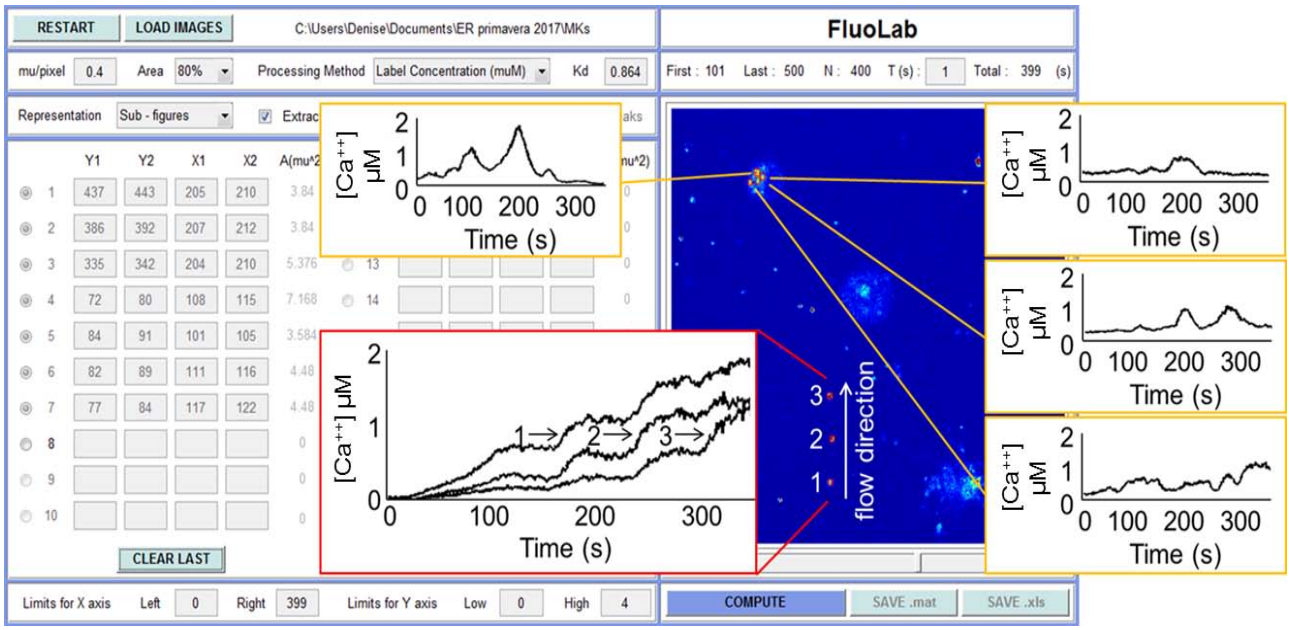

Fig. 1. FluoLab with an example of multi-cell and multi-region contemporary calcium concentration [Ca2+] (µM) extraction

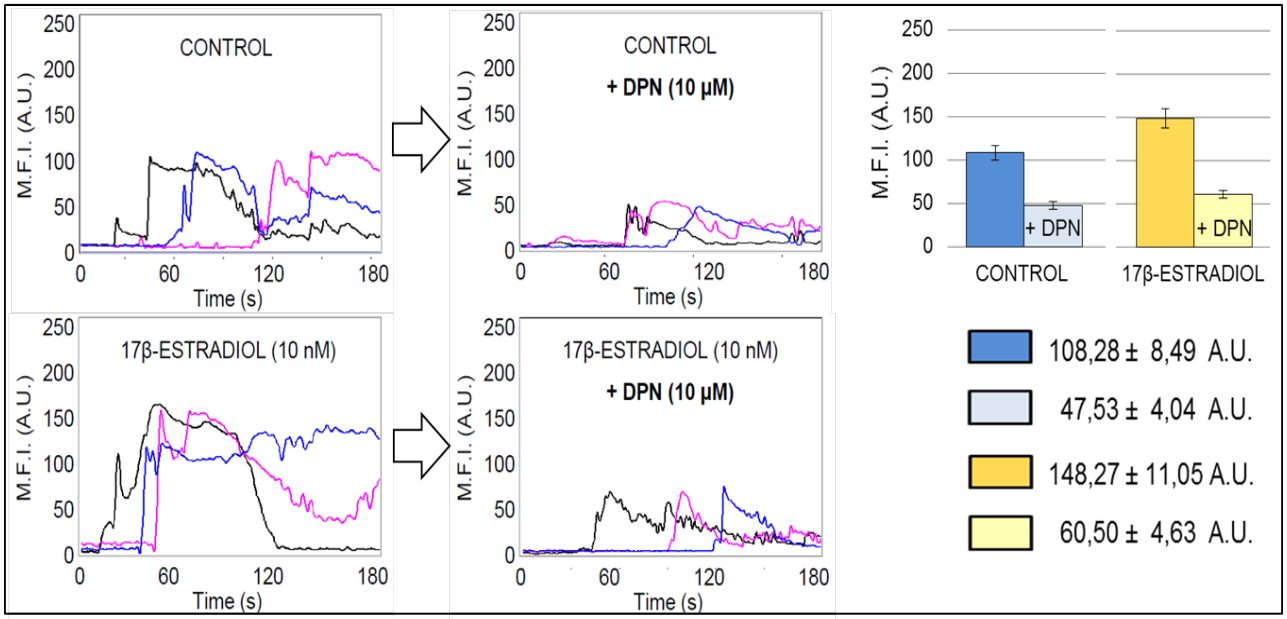

Fig. 2. Example of FluoLab usefulness: investigation and fast quantification of a substance (DPN) effect on calcium functional and metabolic signals, with regard to a research project about estrogens

automatically the background. *FluoLab* allows the calcium signal extraction and analysis in a fast way, step by step in its metabolic evolution. First of all, it computes the calcium concentration combining in a single formula each pixel fluorescence intensity, the maximum and the minimum fluorescence value of each ROI, F_{Max} and F_{min} and the dissociation constant K_d, for each kind of fluorescent probe (FLUO 3-AM, RHOD-1, RHOD-2, TMRM). Then the software allows the filtering of calcium spurious spikes regularizing or smoothing the signals, if chosen. The software leads to the extraction of signals in terms of Mean Fluorescence Intensity (MFI), and converts automatically them in concentration values (µM). The fluorescence intensity variations are monitored from the beginning to end of the analysis period. The data obtained are saved and a file is generated and usable for a fast off-line data elaboration and analysis.

IV. RESULTS AND DISCUSSIONS

FluoLab (Fig. 1) is easy-to-use and it allows a multi-cell functional automatic calcium ion signals analysis. In our laboratory it is fundamental and necessary to discover, discriminate and numerically characterize intra-movements and inter-communications signals, to discover new biological metabolic pathways and to automatize the computation and reduce the processing time.

In Fig. 2 an example of *FluoLab* usefulness is rapidly shown, regarding the extraction of calcium fluorescence signals (expressed as MFI) with the aim of quantitatively investigating a substance (DPN) effect on functionality and metabolism of platelets, with regard to a wider research project about estrogens.

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