Doctoral thesis/dissertation Digest Form

Title of Doctoral Thesis: Miniaturized device for simultaneous imaging and electrophysiology signal acquirement and fluorescence image processing in-vivo and in-vitro

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The study of cells is essential to understand the functioning of living organisms. This study helps to better understand the fundamental units and structures that contribute to an organism's maintenance and homeostasis. Some frequent tools that are used in such studies are fluorescence imaging and electrophysiological studies. Both mentioned methods provide important information regarding the cellular activity and general functioning. Hence, it could be desirable to obtain both signals simultaneously, and determine the relationship between them. However, nowadays there is still no method that can obtain these signals simultaneously. When both measurements take place separately, it is necessary to remove the sample for the optimal condition of an incubator, which can interfere in the best conditions of the experiment. Also, the slightly different position of the sample when positioned for measurement in the different equipment can compromise a reliable interpretation of the correlation between those signals. In this study, we designed and fabricated a compact MEA on a FOP substrate with 25

electrodes of 30 m diameter. The fabrication process employed the standard positive lithography process, and used aluminum and gold films as electrodes. The FOP substrate was used because of its image transmission property. Even with the high noise level obtained by this configuration, we were still able to record and identify spontaneous spikes. The imaging module also proved to be highly satisfactory. The system was designed to be sufficiently compact to fit inside an incubator. With this setup, the simultaneous acquisition of spike activity and fluorescence signals is possible.

Another type of fluorescence imaging that is widely used to better understand cerebral activity is the calcium image. Calcium is a very important ion for cellular and intracellular signaling. One of the main challenges that rises when analyzing such signals is the definition of regions of interest (ROI). Once this area as defined, the calcium signal can be detected and interacting cells identified. Although possible, the task of hand-selecting ROIs can be quite tiresome. Therefore, an automatic method that can accomplish such a task is desirable. In order to obtain an automatic method to define ROIs from calcium imaging data, an algorithm that used a limiar threshold defined by the user was made. For this algorithm, firstly a study about the histogram distribution and PSF was made, as it was import to know if the signal had a mode or not. After defined that it was a non-modal distribution, the threshold value was used to set

clusters of pixels of interest and through the image's deviation and mean, ROIs were finally established. Our algorithm was able to detect almost 50% of ROIs with approximately one σ of the deviation noise. Also, the methodology presented a low number of False Positives.