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An integrated setup for *in-vitro* optogenetic experiments using AI to localize stimulation with a feedback of electrophysiological signals

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INTRODUCTION

Micro-electrode array (MEA):

Many neurobiological studies in the last few decades use the intrinsic electrophysiological properties of neurons. One of the most common techniques within this approach is MEA technology [1]. It is designed both to stimulate and record electrophysiological dynamics of neurons *in-vivo* and *in-vitro*. MEA is a planar array consisting of between a few tens to a few thousands of electrodes (in the case of HD-MEAs), arranged in a matrix and integrated on a chip. The typical material of the electrodes is TiN (or semiconductors in HD-MEAs). The typical diameter of each electrode could reach tens of micrometers and the spacing between them could reach hundreds of micrometers. The electrodes sense the variation of electrical potential in the vicinity due to flow of ions into or out of the cell body. With a designated electronic system, the electrical signals are probed at high sample rate (up to 50KHz), filtered and amplified and provide a global picture of the network activity with high temporal resolution. Another important feature of MEA systems is their capability to provide electrical stimuli to a specific site (via the electrode), which allow the study of evoked neuronal activity. A description of the MEA system is shown in Figure 1.

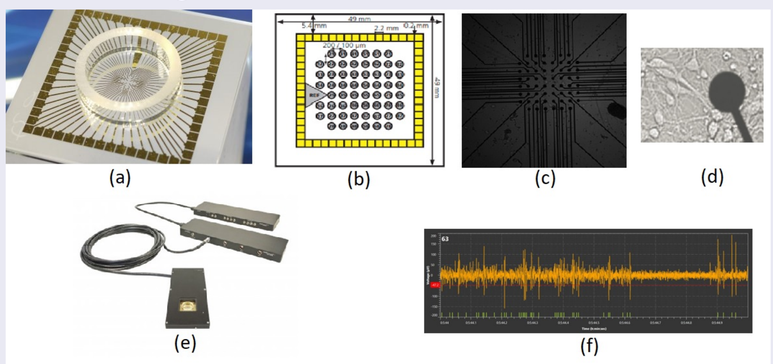

Figure 1: MEA system. (a) MEA chip. (b) Array scheme. (c) Microscope image of the array. (d) A close-up of an electrode surrounded by cells. (e) The electronic system. (f) Signal acquisition and spike detection

Optogenetics:

A novel approach of neuron stimulation, which became widely used in recent years [2]. This technique uses light to stimulate neurons by introducing light sensitive channel protein, Channel-rhodopsin (ChR2), which serves as a light-gated ion-channel. ChR2 is transfected into a neuronal culture and a single neuron can be activated by addressing light with a proper wavelength at the neuron of interest. Figure 2 presents expression of ChR2 by neurons, emitting green fluorescence. Optogenetics is very promising technique since it can easily be localized at specific neuronal cell body, other parts of the cells (such as the axon) or at the neuronal synapses. Using integrated photonics optogenetics can be implemented in compact structures.

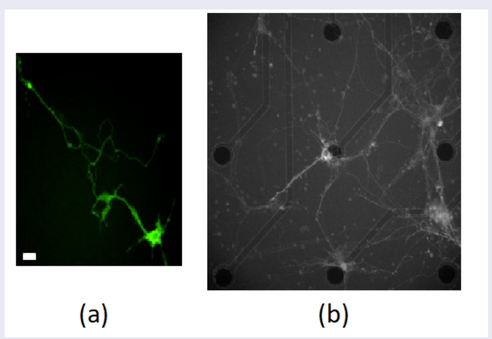

Figure 2: Neurons expressing ChR2. (a) An image of a neuron emitting fluorescence, as a result of an expression of ChR2-GFP, scale bar: 10μm. (b) An image of ChR2 expressing neurons on top of MEA

PROJECT OVERVIEW

The project (acronym *ISLAND*) aims at developing an integrated and intelligent platform which should allow to write and read memories (engrams) in neuronal cultures. The reading part of the setup will be done by sampling the electrophysiological activity of the culture using MEA technology, which is highly time-resolved. The writing part will be executed by optogenetic techniques using an optical setup allowing spatially-patterned light such as digita light processor (DLP) or integrated photonic circuit (IPC). The core of the project will be to integrate those two parts in a closed-loop, by developing processing and control units. The processing unit will collect the electrical signals from MEA as a feedback of the neuronal activity and will map the neurons in the network according to their electrophysiological activity. The control system will then activate the corresponding pattern of stimuli in the optical setup according to the network's map and the assignment. The main idea of the project is depicted in Figure 3.

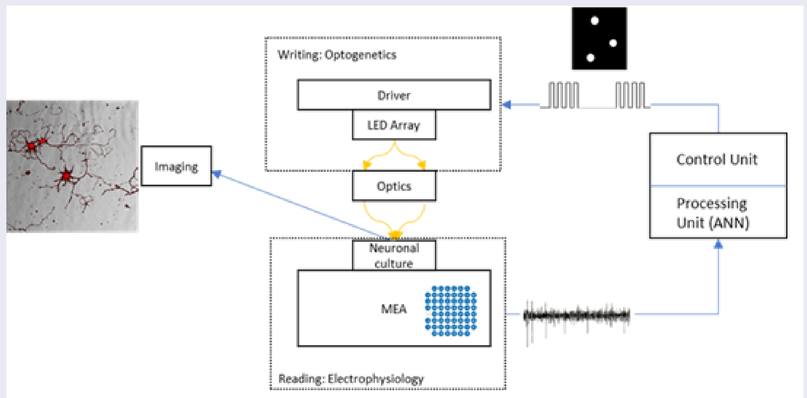

Figure 3: General description of the setup of ISLAND project

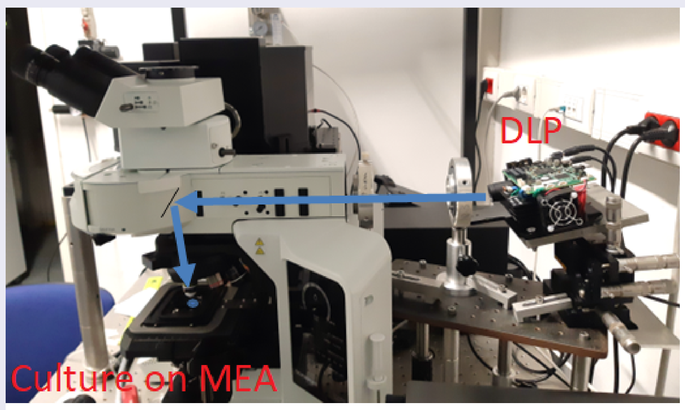

Figure 4: The experimental setup for optical stimulation with DLP

Collaborators:

UNIVERSITÀ DI TRENTO

NanoLab Department of Physics — University of Trento - Italy

Marie Skłodowska-Curie Actions

ISLAND read and write neurons

PRELIMINARY RESULTS AND CURRENT WORK

Optical stimulation and electrophysiological signals standard analysis:

We performed experiments of optical stimuli of the ChR2 infected neuronal culture, using top illumination approach with DLP, as described in Figure 4. The response of the culture was measured by MEA and an analysis of the signals was performed using designated algorithms. Some of the results of the signal analysis can be seen in Figure 5. Among different experiments we performed test of culutre's response to wide-field illumination, spot illumination and long-term-potentiation (LTP) protocol.

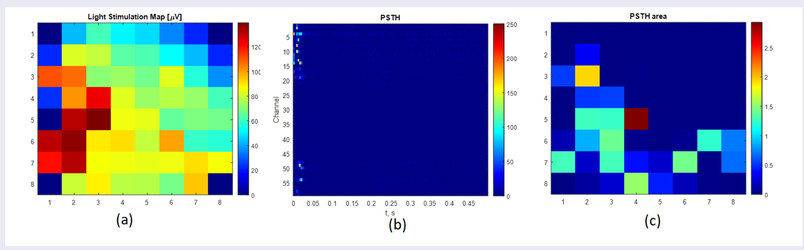

Figure 5: An example of electrophysiological signals post-analysis data. (a) Map of illumination area on top of MEA array. (b) Post-stimulus time-histogram (PSTH)- indicates the neuronal response to light stimultaion for each channel of the MEA. (c) The neuronal response to light stimulation.

Modeling of neuronal networks using Machine-learning tools:

We develop a simplified model which is trained by the recorded MEA data and reproduces the macroscopic network structure of the culture under test (Figure 7). Using the model we can simulate and study the functionality and connectivity of the culture. The model is based on the Reservoir Computing Network (RCN) approach (Figure 6), and will eventually serve as the feedback module in the ISLAND setup.

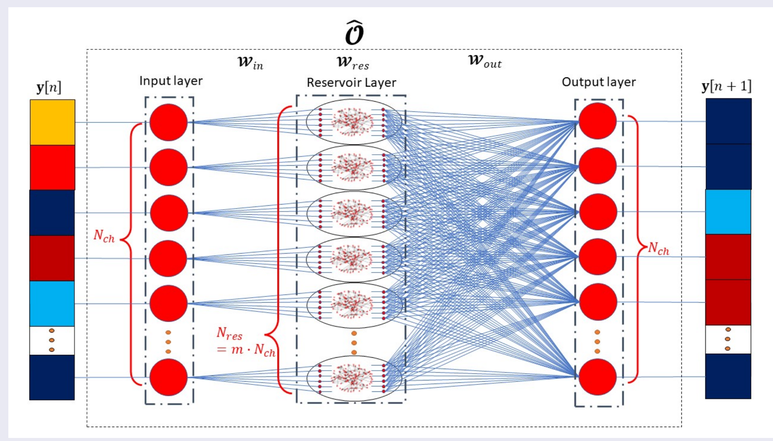

Figure 6: The artificial neural network architecture which is used to model the neuronal network

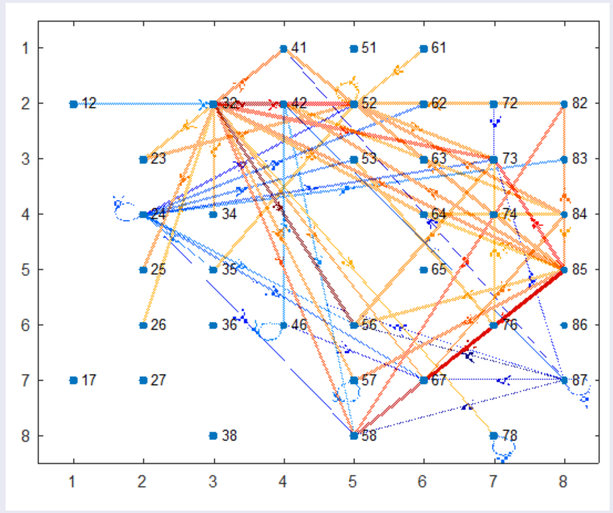

Figure 7: Macroscopic connectivity map of a neuronal network. The map is obtained from the ANN based model

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