IntraCell

ACUTE AND CHRONIC ACTION POTENTIAL RECORDINGS ON MEAS FROM CELO.CARDIOMYOCYTES

FORESEE BIOSYSTEMS

Contents

Introduction

IntraCell is a laser optoporation technology that enables the recording of intracellular Action Potentials (APs) from 2D cardiac monolayers and 3D cardiac organoids on microelectrode arrays (MEAs). The recording of APs with a high signal-to-noise ratio is achieved thanks to the patented laser optoporation technology, which has the extraordinary feature of negligible invasiveness. Cells are not damaged or affected by the process and can be maintained in culture for as long as allowed by the used cellular model. Indeed, laser optoporation and AP recordings can be repeated on the same cells several times over many weeks.

The following protocol describes the full procedure for recording intracellular APs on Celo.Cardiomyocytes from Celogics, (https://www.celogics.com/), using MEAs and acquisition systems from Multi Channel Systems MCS GmbH (https://www.multichannelsystems.com/) (MCS).

Celo.Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes developed under serum-free conditions. They are electrically active and beat spontaneously in both 2D and 3D structures. Their development is focused on electrophysiology, making them ideal for drug-induced electrophysiological studies.

The MCS MEA system allows for recording activity from several types of electrogenic cells plated on optically transparent substrates.

It's worth noting that IntraCell works in combination with all recent MEA systems offered by MCS, including:

- MEA2100-Mini-60-System
- MEA2100-Mini-120-System
- MEA2100-Lite-System
- MEA2100-256-System

This application protocol describes how to manipulate Celo.Cardiomyocytes and obtain APs after acute exposure to drugs and for long-term analysis on MEAs by using IntraCell. The exemplary results were obtained using a MEA2100-Mini-System from MCS, but similar results may be obtained with other MEA systems from MCS always in combination with IntraCell by Foresee Biosystems.

Required Materials

ITEM	PROVIDER	CATALOGUE #
IntraCell	Foresee Biosystems SRL	
FB_Alps	Foresee Biosystems SRL	
Celo.Cardiomyocytes	Celogics	C50
Basal Medium	Celogics	CM200
Advanced Supplement	Celogics	C50-MS
Plating Medium	Celogics	C50-PM
Plating Supplement	Celogics	C50-PS
MEA plates	Multi Channel Systems MCS	
Fibronectin	Roche Applied Science	11051407001
Sterile water	Multiple Vendors	
Phosphate Buffered Saline w/o $\mathbf{Ca}^{2+}$ and $\mathbf{Mg}^{2+}$ (D-PBS)	Sigma-Aldrich	D8537
MEA2100-Mini-System	Multi Channel Systems MCS	
Multi Channel Experimenter	Multi Channel Systems MCS	

Table1: List of materials.

Cell culture

Celo.Cardiomyocytes must be plated on the selected MEA.

For optimal performance, please refer to the following instructions.

Cells Thawing and Media Preparation

For cells thawing and media preparation, please refers to the manufacturer's recommendations.

MEA Coating

1. Place the MEA plate within a 60 mm cell culture dish. Fill a 35 mm cell culture dish with sterile water and place it in the 60 mm cell culture dish, beside the MEA. This will provide a humid environment to prevent droplet evaporation during the coating procedure and cell attachment on the MEA.

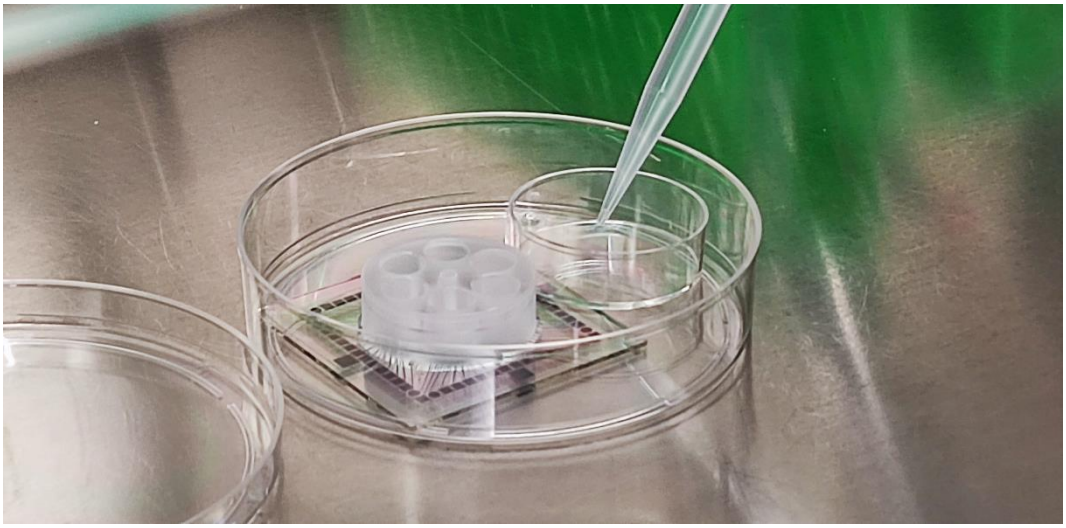

***Figure 1**: System configuration during coating and cell seeding procedure to avoid droplet evaporation.*

2. Dispense an 8 µl/well droplet of 50 µg/ml fibronectin solution over the recording electrode area (central area of the well in which the electrodes are located) of each well of the MEA plate and incubate the MEA plate in a cell culture incubator at 37 °C for 1 hour.

Seeding

1. Foresee Biosystems recommends seeding 10,000 (1 x 10^4) cells on the fibronectin coated area. Calculate the volume of suspension as in the following formula:

$V = \frac{10000}{C}\ x\ W$	V=volume of suspension W=Number of wells to be plated C=number of cells per mL

2. Calculate the total volume of Plating Media required as in the following formula:

$Vp = 5\ \mu l\ x\ W$	V_p=volume of Plating Media W=Number of wells to be plated

3. Dispense a 8 µl droplet of Celo.Cardiomyocytes cell suspension (approximately 10,000 cells) on the centre of the MEA electrodes layout and incubate MEA plate in a cell culture incubator at 37°C, for 1 hour. This procedure is flexible and suitable for all types of MEA devices, ranging from standard single-well up to multiwell plates with 6 or 9 wells. Exemplary MEA plates are reported in Figure 2.

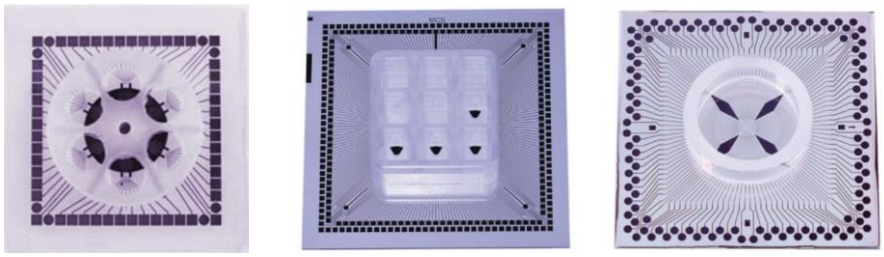

***Figure 2**: MEA devices from Multi Channel Systems GmbH.*

4. Carefully add Plating Medium to the MEA plate (1.5 ml, 350 µl/well, 250 µl/well for standard single-well, 6-well and 9-well plate, respectively) and replace the MEA in the incubator.
5. After 24 hours, replace 90% of the Plating Medium with Advanced Supplement equilibrated in a 37°C water bath. Ensure not to touch or disturb the attached cardiomyocytes.
6. Maintain the cardiomyocytes in the MEA plate and refresh 90% of the spent medium with of Advanced Supplement every 48 hours.
7. Perform MEA recordings between 8 and 10 days after the initial plating.

Baseline recording

When cells acquire regular spontaneous activity (7 days post-plating), the electrophysiological activity can be recorded with the following steps:

1. Take the sample from the incubator and replace 50% of spent medium with the Maintenance Medium following the supplier's protocol; then put it back inside the incubator.
2. Wait at least 2-4 hours after medium replacement, allowing the sample to stabilize.
3. Clean the MEA head stage with pure ethanol.
4. Take the sample from the incubator and mount it on the MEA head stage under sterile conditions.
5. Place a compatible cap on the MEA ring for preserving sterility, as shown in Figure 3.

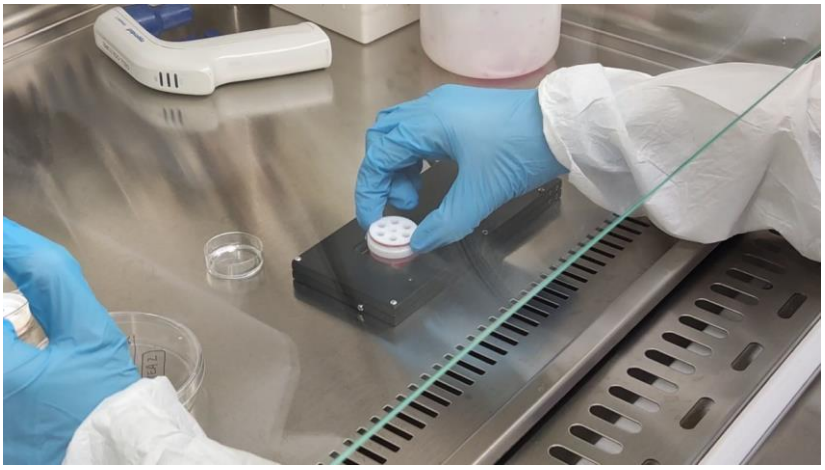

***Figure 3**: the operator places the cap on the top of the MEA. The whole procedure is performed under sterile conditions.*

6. Move the MEA headstage from cell hood and place it on top of IntraCell, as shown in Figure 4. Open the lid of IntraCell and place the MEA headstage in the dedicated socket.

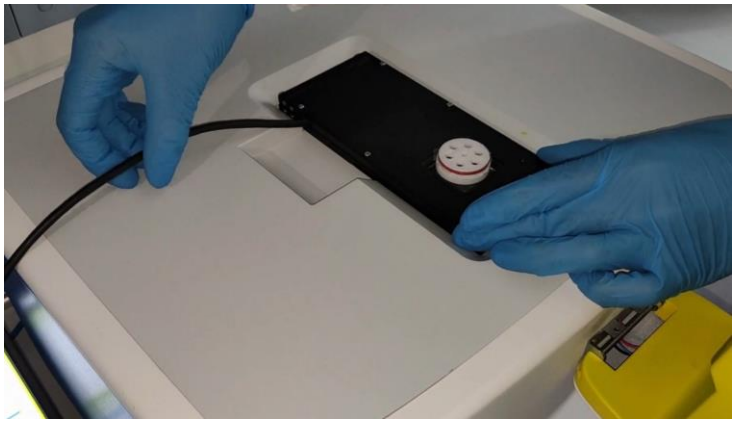

***Figure 4**: The operator completes the configuration of the combined systems: IntraCell plus the MCS MEA headstage.*

7. Close the lid of IntraCell.
8. Open the software FB_Alps on the Computer: the main window will appear on the screen (**Figure 5**). The main window shows the following panels:

a. Information Card
b. Camera View
c. MEA layout and control buttons
d. Laser control panel
e. Scan settings panel
f. Imaging settings panel
g. Action panel
h. Cheat sheet

***Figure 5**: Software FB_Alps: a. Information card; b. Camera view; c. MEA layout and control buttons; d. Laser control; e. Scan settings; f. Image settings; g. Action panel; h. Cheat Sheet.*

9. Via the MEA layout dropdown button, select the MEA layout corresponding to the MEA plate used for plating the Celo.Cardiomyocytes.
10. To adjust the focus: use the Z-bar in the camera view, or ctrl + wheel, and move along the Z-axis to reach the desired focus. Autofocus button can also be used to obtain an automatically focused MEA image. If the user is not satisfied with the result, the focus can also be manually modified after the procedure is completed.
11. Move along the MEA surface in the camera view by pressing Ctrl + Left Mouse or using the X- and Y-bars and set the bullseye (yellow dot) on the reference electrode. Please refer to the IntraCell user manual to know the reference electrodes of each compatible layout.
12. Before starting any procedure, press Set P1 button while on the reference electrode.
    For each well to analyse, press the Autofocus button to adjust the focus, and the Auto-alignment button to set the central electrode. Alternately, press the Whole MEA button to perform the Autofocus – Auto-alignment procedure automatically on the entire MEA.
13. Select the electrodes on the MEA scheme by clicking Shift + Left click. If you want to scan the entire MEA press on Select all.
14. Turn on the Laser using the relative button in the Laser panel.

15. Click on the Scan button to perform laser optoporation on the selected electrodes, note that a successful optoporation is obtained only if the MEA surface is in focus in the camera view. Please remember to start MEA DAQ acquisition from the Multi Channel System software before starting the optoporation process.
16. Use the MCS interface to visualize AP recordings on each electrode selected for optoporation, as shown in **Figure 6**. Please refer to the manufacturer manual for more details.

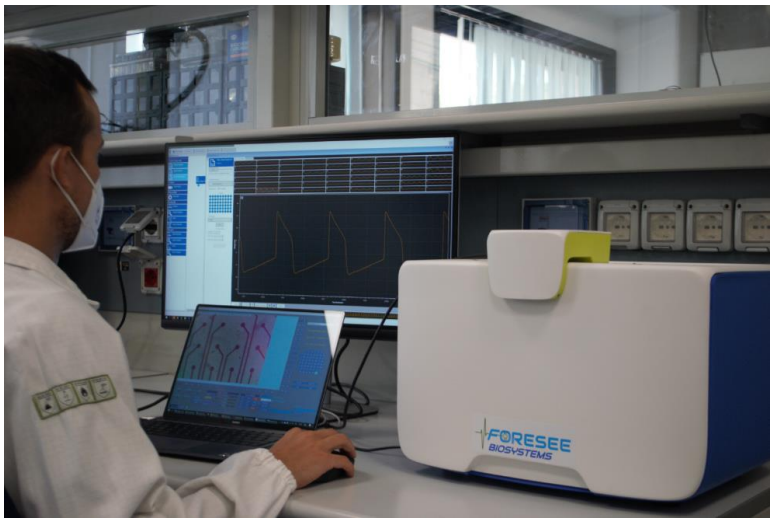

***Figure 6**: the user performs AP recordings exploiting IntraCell and FB_Alps.*

17. (OPTIONAL) IntraCell may be used to optically inspect the cell culture and to acquire images or videos of contracting cells and perform contractility analyses. For further information, please refer to the IntraCell user manual.
18. Other procedures can be found in the IntraCell user manual.

Thus, the user can use IntraCell capabilities to perform single or parallel laser cell optoporation over the electrodes to obtain APs from several cells. Immediately after the laser pulse, transient nanopores are opened on the cell membrane, allowing to switch from field potential to APs with no side effects on the spontaneous activity of the cell, as shown in the exemplary recording in **Figure 7**.

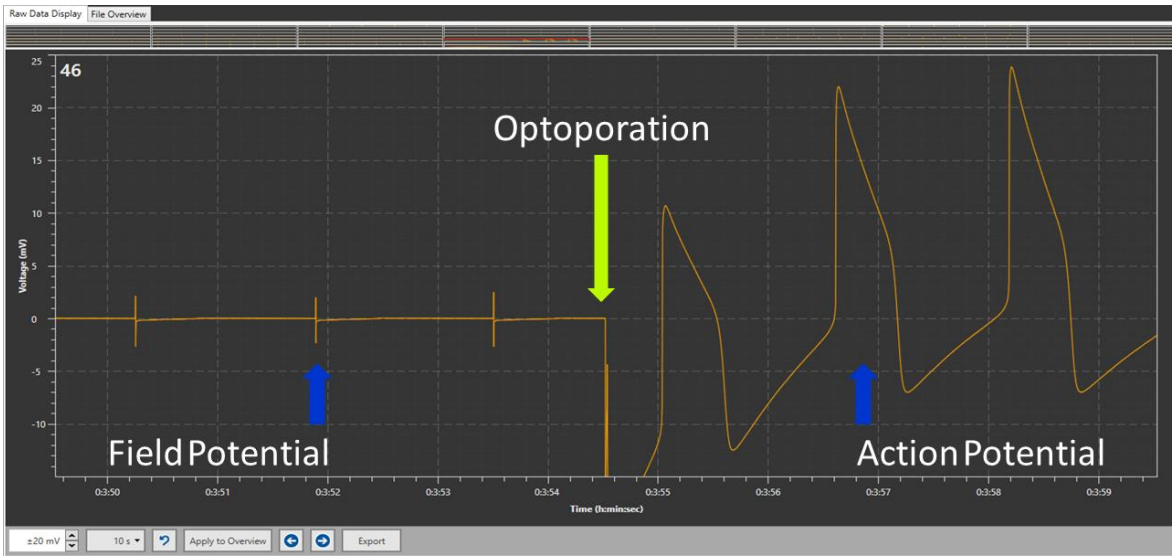

***Figure 7**: transition from the extracellular field potential to the intracellular action potential recording immediately after the laser optoporation.*

Using the automated laser scan technology over the whole 6-well MEA, you will be able to record intracellular APs from each electrode, as shown in **Figure 8**.

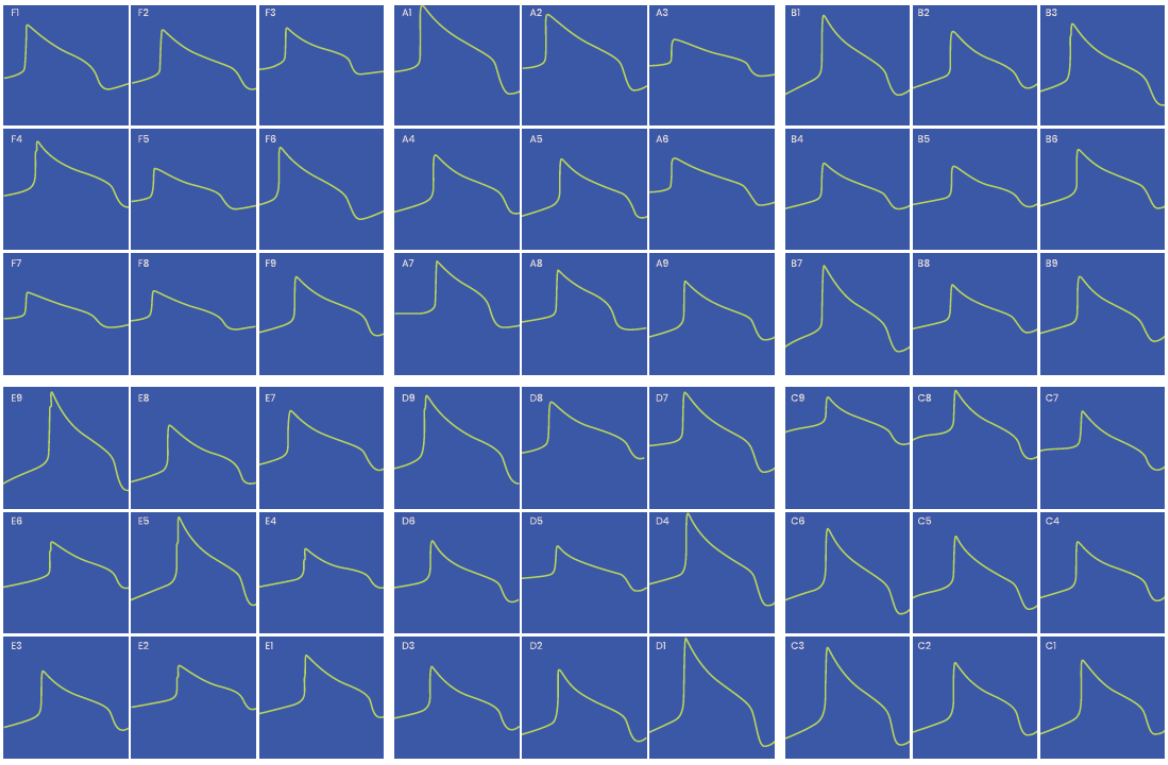

***Figure 8**: AP recordings over the whole 6-well MEA after a complete process of laser scan optoporation.*

Treatment with molecule of interest

1. After the baseline AP recordings, the user can apply the molecule of interest. The user needs to prepare the solution with the molecule, then open the lid of IntraCell and gently inject with a micropipette the solution within the cell culture (**Figure 9**).

2. At this point, it is possible to analyse any potential adverse **acute** effects of the molecule on the cardiac AP. IntraCell allows for precise detection of **AP duration changes** related to drug adverse effects over many cardiac cell models, as shown in Figure 7. AP duration prolongation or Early After Depolarizations (EADs) incidence can be monitored through IntraCell.

3. Once the recording of the desired traces is finished, it is possible to remove the MEA headstage from IntraCell, placing it under a sterile cell hood. Once the MEA device has been removed from the headstage, the user must clean with pure ethanol the glass surface of the MEA device using a paper stripe, to prevent contaminations or removing cell medium that might be spilled on the MEA glass surface. Now, the MEA can be placed back in the incubator. It's important to notice that, at this stage, the drug is still in the culture medium, therefore the long-term culture allows for the monitoring of **chronic adverse effects** on the cells.

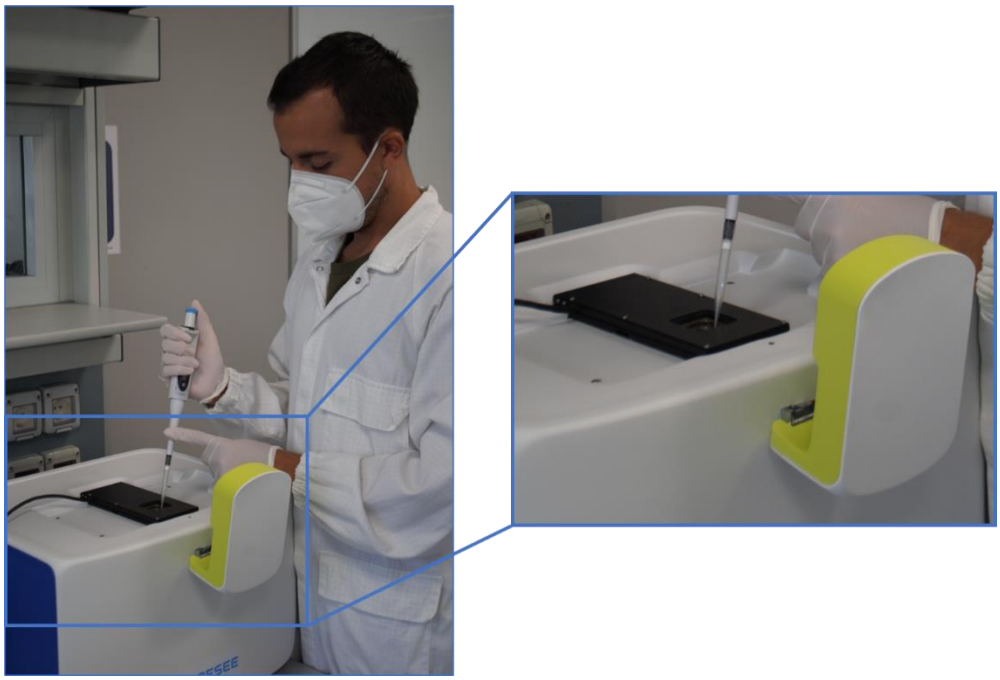

***Figure 9**: injection of molecule within the sample*

Chronic monitoring from the same cell sample

IntraCell is the first tool for monitoring **chronic effects** on AP from *in vitro* cardiac cell culture. Therefore, we developed a protocol of long-term maintenance and AP recordings over many weeks from Celo.Cardiomyocytes. One can repeat the whole process for multiple time-points, depending on type of assays and general cell culture protocols adopted within his/her lab. It is highly suggested to follow the instructions within this application note (see section 4 and 5), to perform optimal electrophysiology experiments. However, the success of the procedure will depend also on the user's skills to handle sensitive cell cultures such as the cardiac ones, critical for example to preserve cell's adhesion on the MEA devices.

Exemplary acute and chronic measurements

In the following sections, we report exemplary acute and chronic measurements in physiological conditions and with molecules that prolong or shorten the action potential of Celo.Cardiomyocytes. All measurements have been obtained with IntraCell from Foresee Biosystems in combination with the MEA2100-Mini-60 from Multi Channel Systems GmbH (Harvard Bioscience).

Acute effects of Dofetilide on Celo.Cardiomyocytes

Figure 10 reports exemplary recordings of Celo.Cardiomyocytes before and after the administration of Dofetilide, a class III antiarrhythmic agent that acts on the delayed rectifier outward potassium current. The represented data are the output of the APP Data Analyzer (https://foreseebiosystems.com/action-potential-profiler-app-2/) software from Foresee Biosystems, which extracts averaged data of the main cardiac cell parameters. The panels on the left represent averaged APs from a well in a 6-well MEA plate. The panels on the right show averaged values of APD30, APD50, and APD90 related to the averaged APs. Measurements with Dofetilide were taken 10 minutes after the administration of the drug. Because of the high repeatability of Foresee laser technology, recordings before and after administration of Dofetilide were obtained from the same cells on the same MEA, without requiring different sample preparations. The shape of the APs as well as the APD values reflect the expected triangulation effect due to Dofetilide. The larger effect occurs indeed on the APD90 duration. The bottom panels show dose-dependent effects on both AP shape and APD values.

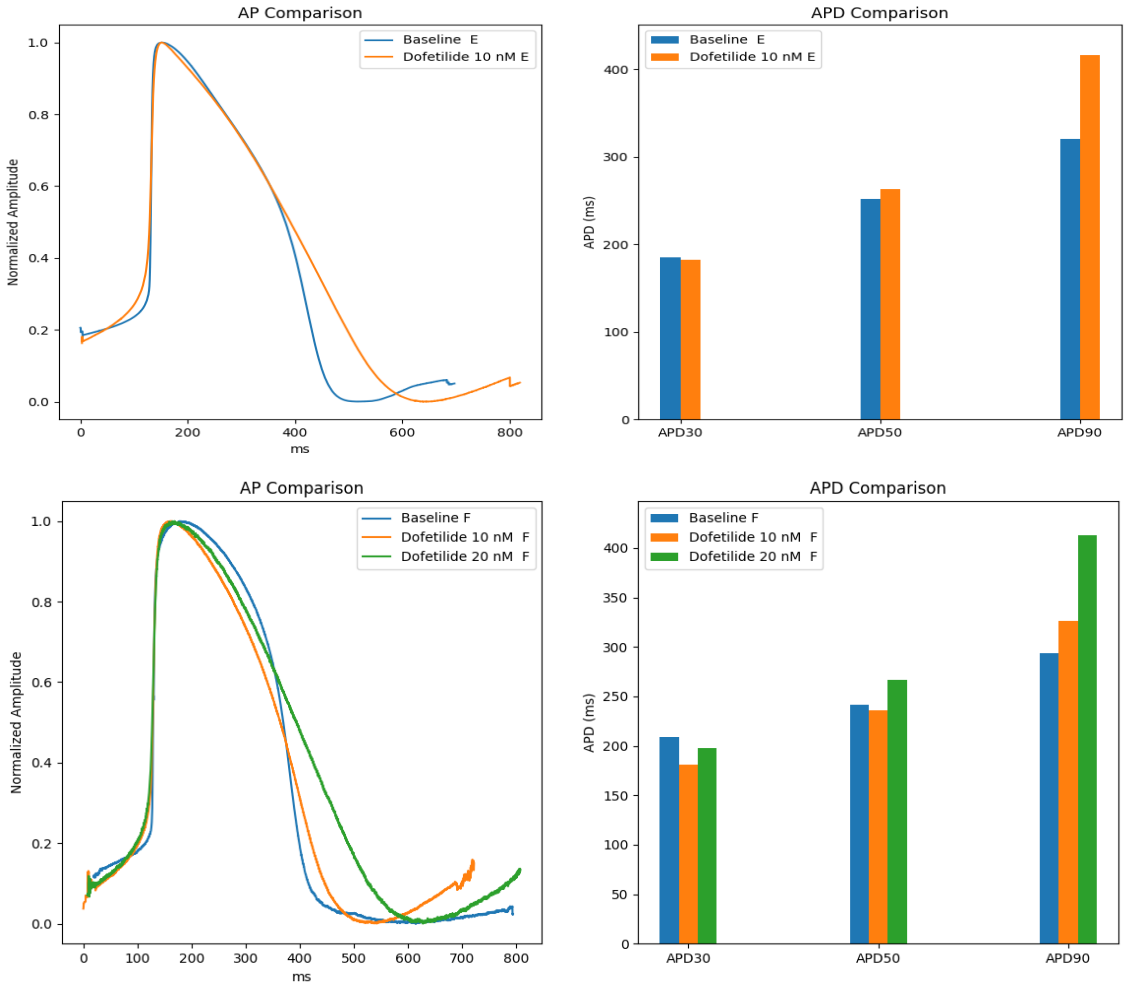

***Figure 10**: Celo.Cardiomyocytes AP before and after administration of Dofetilide at 10 nM and 20 nM concentration.*

Acute effects of Isoprenaline on Celo.Cardiomyocytes

Figure 11 reports exemplary data on the effects of Isoprenaline on Celo.Cardiomyocytes. The datasets at the 2 different concentrations were collected on two independent cell cultures, with the control data obtained from the same cells shortly before the administration of the drug. Data have been analysed and are represented as described in the previous section. The graphs shows that the Celo.Cardiomyocytes respond correctly to Isoprenaline, with a dose-dependent shortening of action potentials.

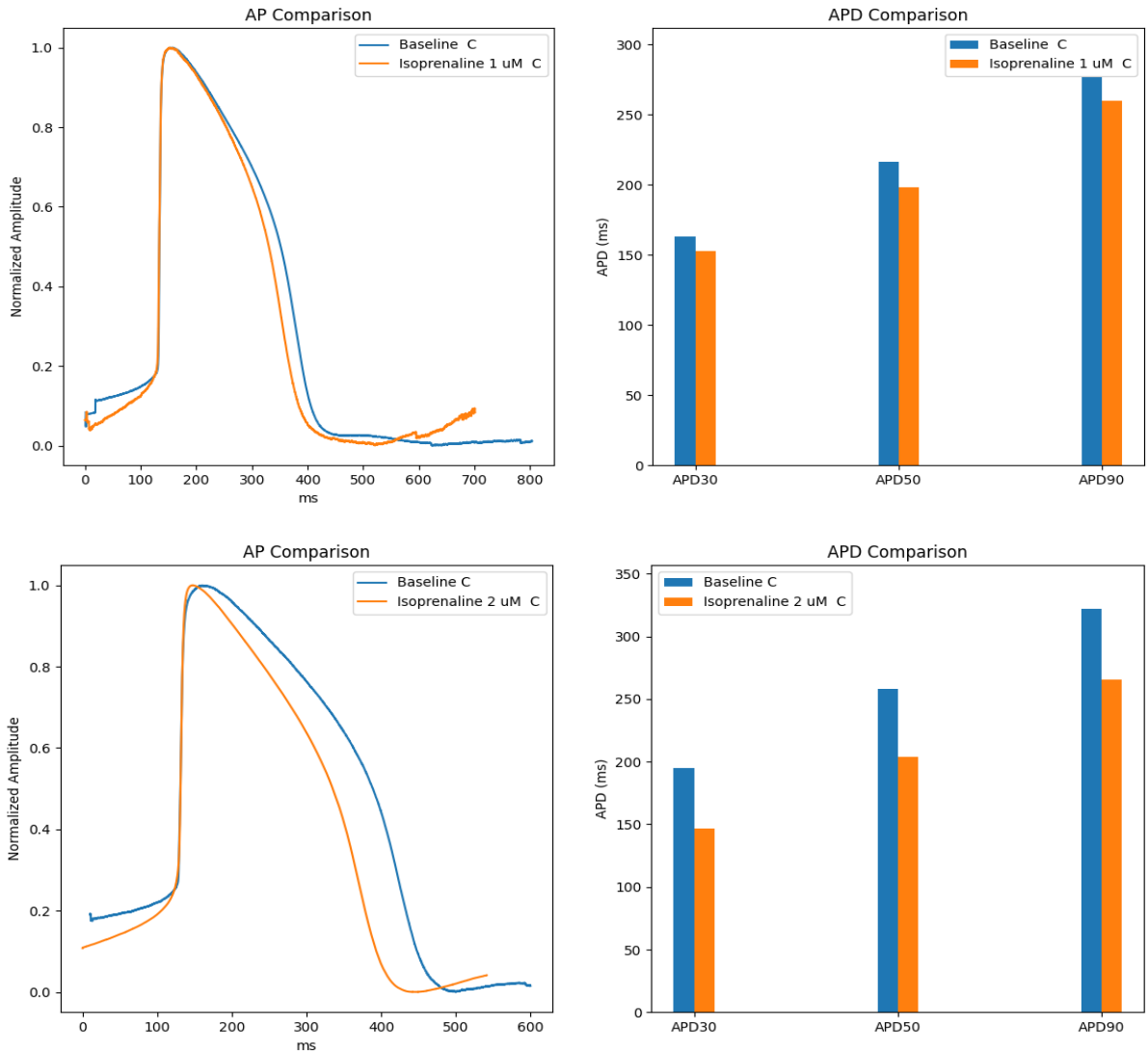

***Figure 11**: Celo.Cardiomyocytes AP before and after administration of Isoprenaline at 1 µM and 2 µM concentration.*

Chronic AP measurements on the same cells

Figure 12 shows averaged APs recorded from the same cell culture over an extended period of 10 days. Cells were measured in five sessions distributed over the 10-day period. This kind of measurement leverages on the non-invasiveness of Foresee's proprietary laser technology for cell optoporation, which does not affect cell health and preserves the cell preparations for consecutive and repeated measurements. The averaged AP shapes and APD values demonstrate the stability of the Celo.Cardiomyocytes over time and the reproducibility of the laser optoporation process, maintaining the cells completely sterile across the various measurement sessions.

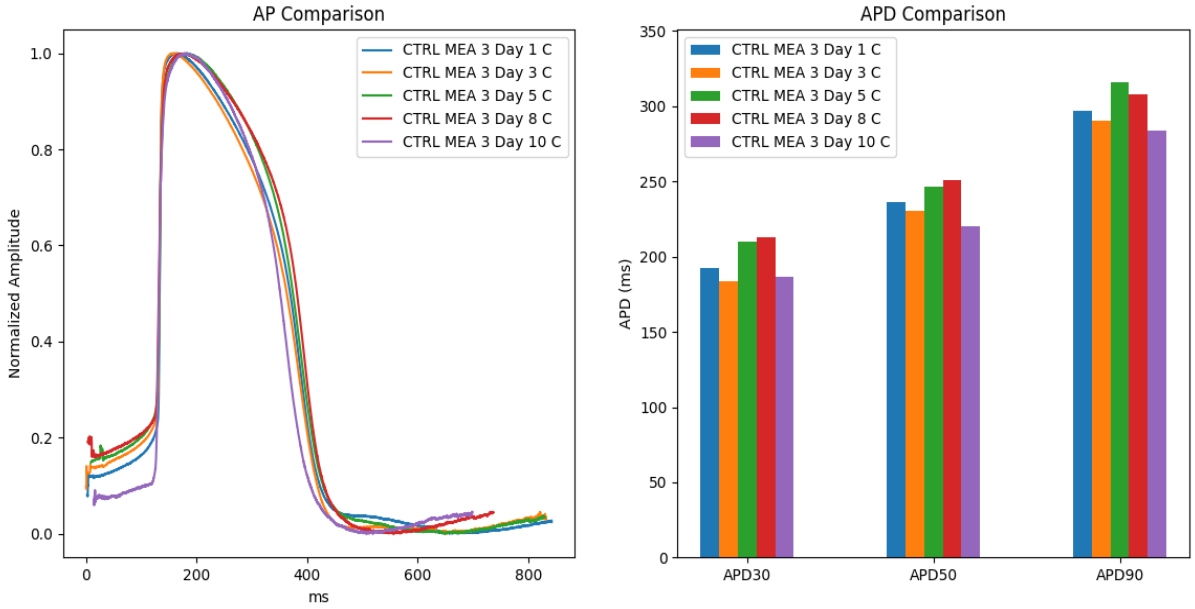

***Figure 12**: Celo.Cardiomyocytes AP from the same cell culture over 10 days' time in vitro.*

AP recordings from 3D Spheroids of Celo.Cardiomyocytes

The cardiac spheroids were generated by seeding Celo.Cardiomyocytes from Celogics cells into ultra-low attachment (ULA) U-bottom 96-well plates (Thermo Fisher 174925) in Plating Medium. A cell density of 2 × 104 cells/well is suitable to obtain spheroids large enough to cover several MEA electrodes. Centrifuge the 96-well ULA plate at 1000 x g for 5 minutes to ensure the cells are collected at the bottom of the wells. 24 hours after plating, replace 90% of the Plating Medium with Advanced Medium. After changing the medium for the first time, the medium should be replaced at 48-hour intervals.
Leaving a small amount of the medium in each well would make sure the spheroids are immersed in the medium. Perform MEA recordings from day 7 post-thawing. The Celo.Cardiomyocytes spheroids can be transferred to a standard MCS-MEA the day of the measurement with the use of a pipette. The procedure to record Action Potentials from 3D spheroids is the following:

- Dispense an 8 µl/well droplet of 50 µg/ml fibronectin solution over the recording electrode area (central area of the well in which the electrodes are located) of each well of the MEA plate and incubate the MEA plate in a cell culture incubator at 37 °C for 1 hour.
- Under the sterile hood, take the 3D spheroid from the ultra-low attachment (ULA) plate with a pipette.
- Gently place the 3D spheroid on the active area of the MCS-MEA, namely on the electrodes area.
- Add just enough cell medium to cover the 3D spheroid and the electrodes (including the MEA reference electrode). Adding too much cell medium may result in the 3D spheroid floating away from the electrodes.
- Mount the MEA with the 3D spheroid into the MEA headstage placed on IntraCell.
- Apply laser poration with IntraCell to enable the recording of intracellular Action Potentials from the 3D spheroid. **Figure 13** shows exemplary Action Potentials recorded from a 3D spheroid of Celo.Cardiomyocytes.

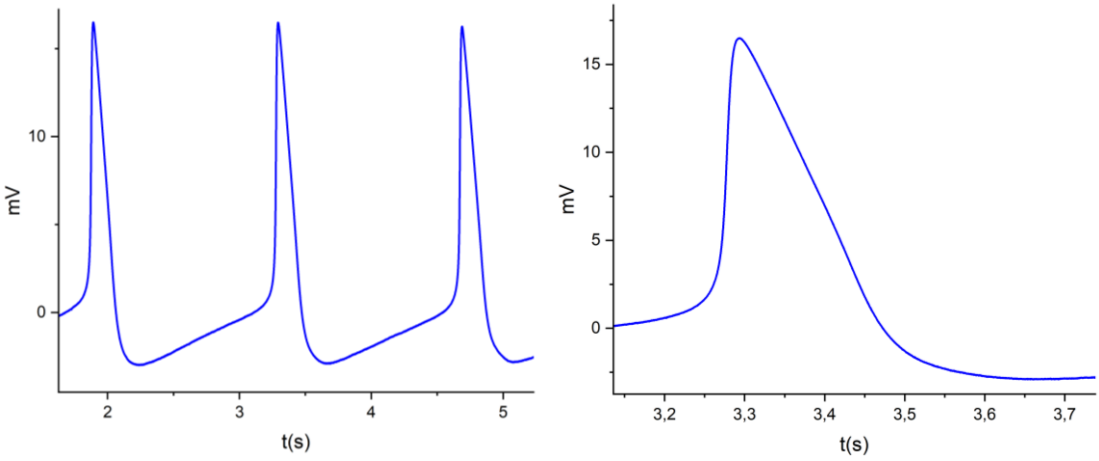

***Figure 13**: AP recordings from Celo.Cardiomyocytes 3D spheroids.*