MEABeat: a software for enhanced electrophysiological analysis of hiPSC-derived cardiomyocytes using Multi-Electrode Arrays

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***Abstract*—Cardiovascular diseases are ranked as the leading cause of mortality worldwide. One promising method for gaining insights into cardiac malfunctions is through the use of human-induced Pluripotent Stem Cells-derived cardiomyocytes (hiPSC-CMs). These cells exhibit contractile features and gene expressions akin to typical cardiomyocytes. Due to their electrophysiological properties, hiPSC-CMs are well-suited for evaluation with multi-electrode arrays (MEAs), which detect the extracellular field potential in contracting cardiomyocytes attached to substrates. This detection mirrors the transmission of cardiac action potentials across the cell layer. This research presents *MEABeat*, an innovative software developed for the analysis of hiPSC-CM signals. *MEABeat* offers a more adaptable experience than traditional software, designed to accommodate diverse analysis needs for cardiac researchers. As a demonstration, it was applied to signals garnered from experiments on drug application, maturation tracking, and stability assessments of hiPSC-CMs. The software is freely available for academic use.**

***Keywords*—signal processing, cardiac signals, hiPSC-CMs, MEA**

I. INTRODUCTION

Given the increasing adoption of human-induced Pluripotent Stem Cell-derived cardiomyocytes (hiPSC-CMs) for modeling cardiac diseases and evaluating therapeutic interventions, there is a growing demand for more advanced tools capable of analyzing the complex electrophysiological signals these cells generate. hiPSC-CMs, which closely mimic the behavior of native cardiomyocytes, exhibit contractile activity and electrophysiological characteristics that can be precisely recorded using Multi-Electrode Arrays (MEAs). MEAs are widely recognized as powerful tools for long-term, non-invasive monitoring of extracellular field potential signals, providing insights into key cardiac processes, including action potential propagation and arrhythmic behavior [1] [2].

Although existing MEA analysis software, such as *MC_Rack* by Multi Channel Systems, offers several essential features—such as signal filtering, triggering electrode selection, and data visualization—it has notable limitations when applied to hiPSC-CM recordings. Originally designed for neuronal spike detection, *MC_Rack*'s spike-centric framework makes it less suited to cardiomyocyte signal analysis. Moreover, while *MC_Rack* provides reliable visualization and some baseline analysis, it lacks the flexibility to perform more complex, user-defined comparisons and numerical evaluations across different experimental conditions. This limitation becomes particularly evident in studies that involve drug testing, maturation tracking, or stability assessments of hiPSC-CMs.

To address these gaps, we implemented *MEABeat*, a new software tool specifically designed to handle the unique properties of cardiomyocyte-derived MEA signals. Unlike existing platforms, *MEABeat* emphasizes user-friendly operation, flexible data processing, and customizable analysis workflows, enabling researchers to compare baseline signals with those obtained after experimental interventions more effectively. By generating quantitative results tailored for efficient statistical evaluation, *MEABeat* facilitates a deeper understanding of hiPSC-CM behavior under various conditions. The software was tested on real-world data, including signals from cells subjected to drug treatments and long-term culture, and was developed in collaboration with the IRCCS San Raffaele Hospital, where the hiPSC-CMs were cultured and recorded using Multi Channel Systems' equipment.

Thus, the development of *MEABeat* represents a critical step toward more sophisticated and adaptable tools for cardiomyocyte signal analysis. By overcoming the current limitations of *MC_Rack* and offering a more specialized platform for hiPSC-CM studies, our software has the potential to accelerate discoveries in cardiac research, improve reproducibility, and support the development of novel therapies for cardiovascular diseases.

The extracellular signals recorded from hiPSC-CMs closely resemble the clinical electrocardiogram (ECG) waveforms; therefore, in this work, the nomenclature will refer to the distinct waves characterizing the field potential as *pre-potential* for the initial positive wave preceding the most prominent peak of the field potential, in turn, defined as part of the *QRS complex*, and finally as *T peak* for the wave that follows it.

MEABeat is freely available for academic use, with open-source code and documentation provided at https://github.com/francescodesantis/MEABeat.

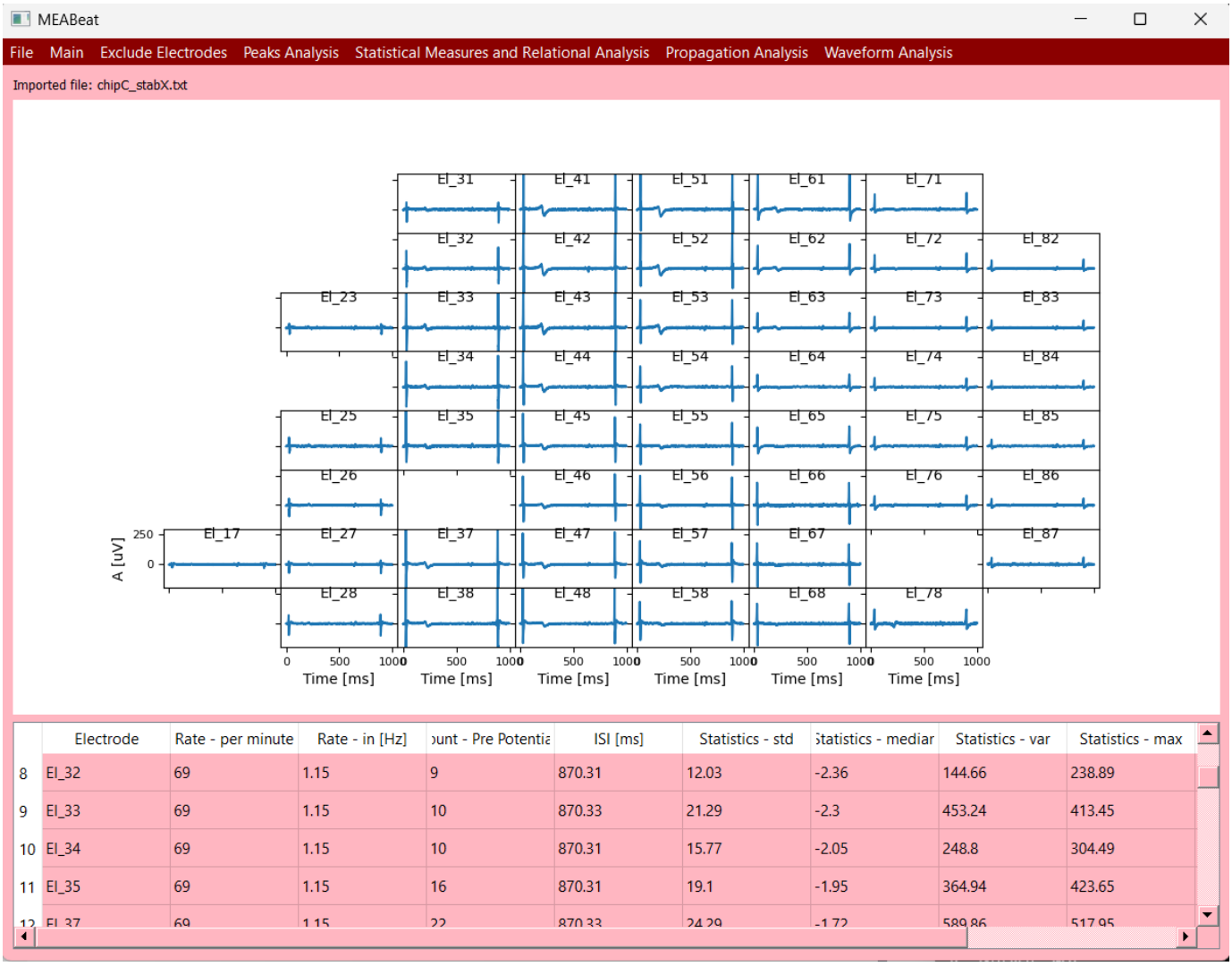

Fig. 1: Peaks analysis and Statistical measures conducted on non-excluded electrodes, with the results displayed within the user interface.

II. Materials and Methods

A. Recording System

The employed MEAs are Standard MEAs with 60 electrodes placed in an 8x8 grid, and an internal reference (iR) electrode connected directly to the amplifier's ground. The recording setup was composed of an amplifier (MEA1060-Inv-BC), a data acquisition device (USB-ME64), a power supplier (PS40W) and a temperature controller (TC02).

B. MEABeat

An overview of the software's key functionalities is now provided.

1) Key Features:

Data Import. Users can import .txt files, which are generated by converting .mcd recordings (a proprietary file format) into ASCII format. During this import process, electrode data and names are extracted, organized, and saved with the initial time and amplitude values for subsequent retrieval. To ensure precise peak detection, signals are resampled at 1 kHz only if the original sampling rate exceeds this value.

Data Visualization. Within *MEABeat*, users can visualize both raw and filtered data arranged in a grid of subplots, presenting only the initial thousand data points and adhering to the typical layout for square grids (Fig. 1). Double-clicking on a subplot causes it to expand, revealing the complete signal as well as a Navigation Toolbar and a *Compute Distance* button that facilitates the measurement of x and y-axis distances.

Exclude Electrodes. Two options are provided: *Manual Exclusion*, which allows users to deselect electrodes by their name, and *Frequency-based Exclusion*, prompting the user to specify a beats-per-minute (bpm) threshold. This feature is crucial for removing irrelevant and noisy signals from the analysis.

2) Analysis Tools:

Filtering. A careful analysis of the literature regarding the

filtering of cardiac signals, mainly ECG, led to the choice of the following filters in this sequence: Savitzky-Golay filter, adaptive Butterworth filter and moving average filter [3]. This sequence was selected based on literature and empirical evaluations as a suitable compromise to balance signal preservation and noise reduction. However, we acknowledge that filtering remains a trade-off, and that non-linear phase effects—especially with the adaptive Butterworth filter—can affect waveform integrity.

Peak Detection The R peak locations must be computed for each signal to carry out the data analysis, which is accomplished using `find_peaks` function from Scipy.signal module. In `find_peaks`, a peak or local maximum is defined as any sample whose two direct neighbours have a smaller amplitude. In order to improve peaks detection, the variable of *prominence* is added as input of the filtering panel. The *prominence* of a given peak is defined as a measure of how much a peak stands out from the surrounding baseline of the signal, rather than considering the absolute zero.

Peaks Analysis. The identified peaks facilitate various standard analyses, such as determining the number of pre-potentials detected and calculating rate values expressed in bpm and Hz. Furthermore, the average RR-interval (RR) can be derived as the average time difference between each successive peak across the entire signal. Plotting pre-potentials involves displaying them on the expanded subplots.

Statistical Measures and Relational Analysis. Several statistical metrics are calculated for significant signal portions around the R peaks: standard deviation (std), mode, median, variance, minimum, and maximum values. The *Relational Analysis* encompasses Cross-Correlation and Transfer Entropy between two signals. For standard Cross-Correlation analysis, two measures are available: the linear correlation coefficient (Pearson Coefficient) and the cross-correlogram. The Pearson Coefficient provides a numeric representation of the linear relationship's strength and direction between two signals, whereas the cross-correlogram delivers a detailed view of their correlation over varying time-lags. Additionally, *MEABeat* supplies another method to measure Cross-Correlation [4], defining a temporal proximity relationship between two selected signals by measuring how often spikes from both occur simultaneously or within the same time window. Furthermore, Transfer Entropy identifies information flow between two signals, showing whether one electrode's activity is affected by another.

These measurements depend on the choice of a reference electrode, so the software records the order in which the two electrodes are selected for analysis, and an analysis results window is presented.

Propagation Analysis. Propagation Analysis offers a dynamic tool for illustrating interactions within MEA electrodes. Upon activation, a dialog box prompts users for two parameters: *correlation window* and *occurrence threshold*. The *correlation window* specifies the interval around the reference peaks within which the time-lags of other electrodes are calculated for each beat, while the *occurrence threshold* excludes electrodes with peak counts below this threshold compared to the maximum peak count. The time-lags are measured against the reference electrode, identified as the one with the highest R peak count, and the averages are presented in a table, with an asterisk denoting the estimated pacemaker, the earliest activating electrode. Users can save this table as an Excel file for further examination or opt to view the associated propagation heatmap (Fig. 2). If they choose the latter, the computations are reiterated concerning the pacemaker, allowing visualization through a heatmap that updates continuously with each peak computation. The estimated pacemaker is marked, while electrodes lacking any R peak within the designated interval around the reference peak are removed from the visualization.

Waveform Analysis. Morphological analysis is performed on the averaged signals to minimize noise and extract the desired waveform. Prior to signal averaging, the mean RR is calculated to establish a dynamic window centered around the R peaks. Subsequently, the complexes are aligned and averaged on a point-by-point basis. The `find_peaks` function detects the R peaks, the R onset (equivalent to the Q peak in a standard ECG signal), as well as the S and T peaks. For proper alignment with the displayed averaged signal, peaks of a specific wave are chosen, and the complex start is subtracted from the indices of these peaks.

The analysis of averaged signals provides the calculation of the Peak-to-Peak measurement, T wave amplitude through various techniques, Field Potential Duration (FPD) using the Trapezium's Area method [5], and its correction using Fridericia's formula [6]: $FPD/[RR/1000]^3$.

3) Results Handling:

Results. Analysis findings are presented in a table within the GUI for all electrodes not excluded from the study. Users have the option to export this table as an Excel file.

Comparison of results. Users can compare two Excel files by examining shared parameters and electrode identifiers. This comparison highlights the mean value for each applicable parameter across all relevant electrodes, allowing a swift evaluation of the data. These mean values can be saved into an Excel file for comprehensive comparative analysis. This feature is accessible at any point, making *MEABeat* a valuable tool for comparison purposes even without further analyses.

III. Results

A. Validation of MEABeat

We validated MEABeat's performance through both visual inspection and quantitative comparison of key features (e.g., peak detection, frequency in Hz, rate in bpm, peak-to-peak amplitude, and field potential duration). Results from four electrodes across five recordings were compared to those obtained with $\mathrm{MC}_Rack. The computed mean frequency and rate were nearly identical,$

B. Results of experiments

Stability Tests assessed how hiPSC-CMs react to various environmental factors, and Maturity Tests revealed their

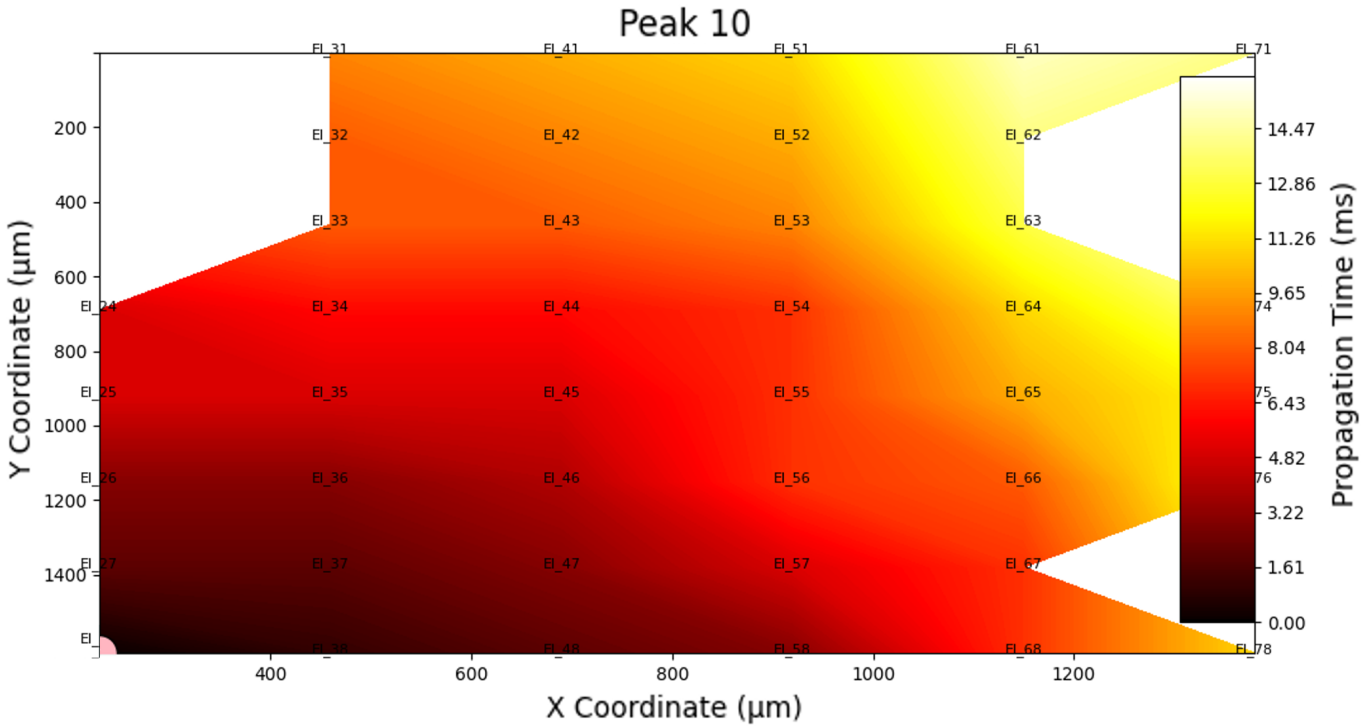

Fig. 2: Propagation heatmap continuously updating at each peak, here shown for the 10th signal peak. A colorbar is present, representing the time-lags in ms characterizing the electrodes spiking activity with respect to the reference electrode. The X and Y coordinates are referred to the MEA measurements.

temporal behavior, which is crucial for subsequent analyses and experiments. Drug Tests with Verapamil, a Ca^{2+} channel blocker, showed the anticipated decrease in the beating rate, eventually causing arrest at higher doses. Stimulation Tests confirmed that hiPSC-CMs naturally paced without the need for additional electrical stimulation.
Typically, experiments are carried out on cells that exhibit stable behavior after several days of maturation and after refreshing the culture medium.

IV. Conclusions

In summary, *MEABeat* provides an efficient and versatile platform for the analysis of hiPSC-CM signals obtained from MEAs. Its graphical interface has been iteratively refined based on informal feedback from several researchers, though no formal usability study has yet been conducted. Consequently, *MEABeat* facilitates diverse analytical workflows, promoting progress in cardiac electrophysiology and serving as a basis for novel applications in cardiac research.

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