Low-cost voltage amplifier for biological signal acquisition through generic micro-electrode array

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***Abstract* — This paper presents a generic voltage amplifier intended to be used in a platform for acquisition of electrophysiological signals through a multi-channel micro-electrode array (MEA). The design is compatible with commercially available MEAs and is intended to be used in any experimentation platform with multiple parallel channels for signal acquisition. The design and the preliminary experimental results, which confirm the feasibility of the design, are presented.**

Keywords: amplifier; biological signal; acquisition; Microelectrode array

I. INTRODUCTION

The field of cellular study has advanced since its inception [1], where scientists were limited to passive observation of single cell organisms or cellular tissue through simple microscopes [2]. Nowadays, a plethora of new options are available when undertaking cellular studies, and one of those options is the study of the electrical characteristics of groups of cells, the study of cell-generated voltage spikes, their transient response during voltage and other electric excitation [3], and their response to external stimuli [4]. A very useful tool developed to help with this kind of studies or experimentation are microelectrode Arrays [5].

Micro-electrode arrays (MEAs) are devices in which several tens of micrometer sized electrodes that are connected to external circuitry are arranged in a medium suitable for the growth and nurture of different dissociated cell cultures [6] or tissue slices [7], in order to be able to electrically interface with said cells or tissue, be it for reading and interpreting their states through analysis of their electrical response, exchanges and cellular dynamics, or for influencing them through a wide manner of stimulus [4][8].

In order to make good use of precise measuring and cell interfacing tools such as micro electrode arrays (MEAs) it is necessary to amplify the signal that the MEA outputs to a range where common recording tools such as 12 bits ADCs, which have a resolution of 1.22mV for a voltage supply of 5v, can pick up and record the signal clearly, while maintaining a good SNR, in order to make the interpretation of recorded signals easier for cellular specialist trying to extract information out of them.

Although readymade solutions for this problem already exist, commercially available MEA amplifiers usually cost upwards of thousands of euros and using other types of untested amplifiers can lead to distortions or attenuations in the shapes of the desired signals [9], so it was decided that a new amplifier would be developed in house while adhering to a few basic principles: reliability, low cost, and versatility for a wide range of uses.

The signals expected to be yield by the MEA in these experiments vary between 50 to 100 microvolts, to which a gain of 5600, or about 74.96 dB, will be applied with our amplifier, while filtering the signal to reduce unwanted noise. This is done to leave a certain room to record bigger signals and spikes in case that they occur without saturating the amplifier, so that no loss of information takes place, such as the total amplitude of the spike, and try to reduce noise to a minimum.

In the following sections, the design process for the amplifier will be explained in detail, along with the justifications for every decision taken while designing the amplifier, in order to make the process clear for the reader. Following that, experimental results will be shown in order to show the capabilities of the amplifier while testing with inputs coming from simulators and real in vitro cells.

II. DESIGN

In order to obtain voltage gains of the desired magnitude, multi-stage amplifiers are typically used [10], including band-pass filters to reduce the final noise, due to the fact that normal operational amplifiers are not capable of providing such ranges of amplification, or are barely capable but cannot maintain a stable frequency response, which is needed in order to not alter the recorded signals in its intended recording range, which takes place between 1 Hz to around 4 kHz. Any kind of distortion could easily misrepresent the total amplitude of certain peaks of signals, or completely deform some transient responses which are useful in the study and interpretation of cellular activity.

The amplifier consists of a first stage with a gain of 100 times, as physically close to the MEA as possible to reduce trace lengths in order to decrease any possible electromagnetic interference induced in the traces or any of the connecting wires, which also will be kept as short as possible. Afterwards, the signal is filtered by a second order passive band pass filter in order to eliminate undesired noise from the signal and any possible dc shift that may occur due to the first stage amplifier.

Lastly, the signal is fed onto the second and last amplifier stage for the signal to reach its desired range, which will then be recorded by a common 12-bit ADC in a SMT32-L476RG microcontroller.

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It can be observed in Fig. 1, representing the amplifier circuit diagram, that the main operational amplifier for the circuit is a TLC074 (Texas Instruments).

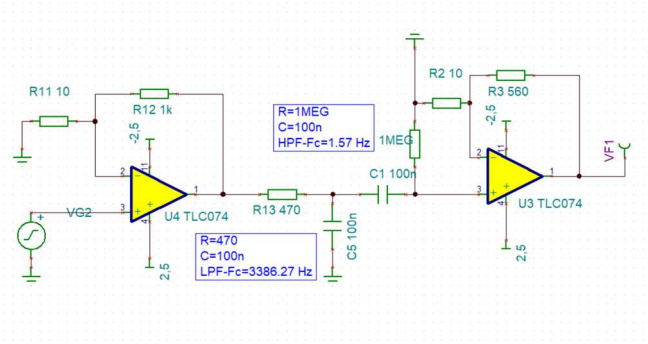


Fig. 1. Amplifier circuit schematic

This decision was made after cross testing it against other available amplifiers at the time of design, among which precision amplifiers such as the INA333 (Texas Instrument). The TLC074 demonstrated experimentally that it was less prone to amplify noise, leaving a cleaner signal when tested with the same samples at the same gain. Although the TLC074 will induce a variable dc shift level after the first stage, that will be removed by the high pass filter that comes right after it.

After extensively studying the TLC07X amplifier family datasheet, two main points have been extracted to take into consideration when designing our circuit.

The first one is to provide a return path for the input bias current, which is not a problem for the second stage, but in order to not alter the signal at the first stage [9], the circuit will have to deal with the dc shift caused by de input bias current. This problem will be solved with the high pass filter component of the band pass filter located between the first and second stages.

The second one consists of a limitation of the maximum values of the feedback resistor, which the datasheet limits to 5 kΩ at maximum, due to the addition of a pole to the transfer function equivalent to the input capacitance multiplied by the combination of source resistance and feedback resistance. In order to mitigate this effect, if the resistance must be higher than 5 kΩ by design constrains, a capacitor could be added in parallel, but that is not needed in this application.

In order to filter out unwanted noise and try to maintain a good SNR, a second order passive band pass filter is added to the circuit immediately after its first stage. It is composed by two first order passive filters, whose cut-off frequency, after the study of other commercially available amplifiers, has been determined to be around 1 Hz for the high pass filter and around 3kHz for the low pass filter, in order to be able to experiment with different local field potentials, which are transient electrical signals generated in cell tissues by transient imbalances in ion concentration in the spaces outside cells, with the same hardware.

The values of the components that compose both filters are calculated by introducing a normalized capacitor value of 100 nF into the cut-off frequency formula (1) for this type of filters:

$$f_{-3dB} = \frac{1}{2\pi RC} \qquad (1)$$

With this equation, it can easily be solved for the desired frequencies, which results in a low-pass filter with a cut-off frequency of 3386.27Hz if a 470 Ω resistor is chosen, and a high-pass filter of 1.57Hz if a 1MΩ resistor is selected.

The last part of the design process is to select the resistor pair for the amplification stages, which will set their gain. Knowing that a total amplification of around 5000 to 6000 must be reached, the first stage will be fitted to have a gain of approximately 100, while the second stage will have a gain of around 56, in order to use normalized value resistors. Using the gain formula (2) for the non-inverting amplifier configuration, which is the one used on this amplifier circuit as shown in Fig. 2:

$$Gain = 1 + \frac{R_{feedback}}{R_{in}} \qquad (2)$$

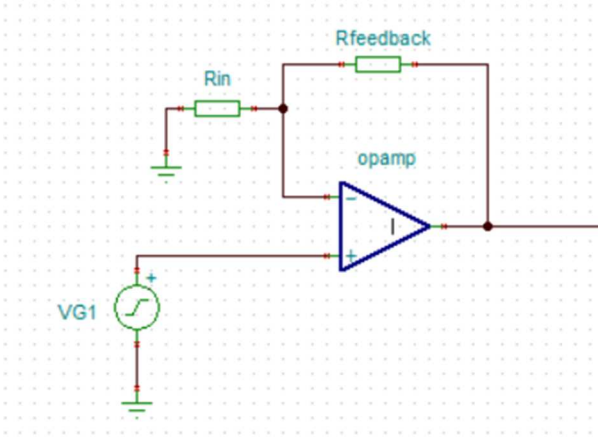


Fig. 2. Gain formula reference.

And taking into consideration the fact that the TLC074 datasheet discourages using a value greater than 5kΩ for the feedback resistor, the final resistor values chosen for both stages are a pair of 10Ω and 1kΩ for the first stage and 10Ω and 560Ω for the second stage.

III. Experimental Results

The first experiment performed on this amplifier was the amplification of different common wave forms, such as sinusoidal, sawtooth, and square signals, with an amplitude of 100 µV in order to not saturate the amplifier, while varying their frequency in order to study the frequency response of the amplifier. This was done in order to experimentally determine the real Bode diagram of the amplifier by varying the frequency of the input signal. The result, which can be seen in Figure 3, greatly resembles its theoretical Bode modelled using TINA, a PSpice-based simulator [11]:

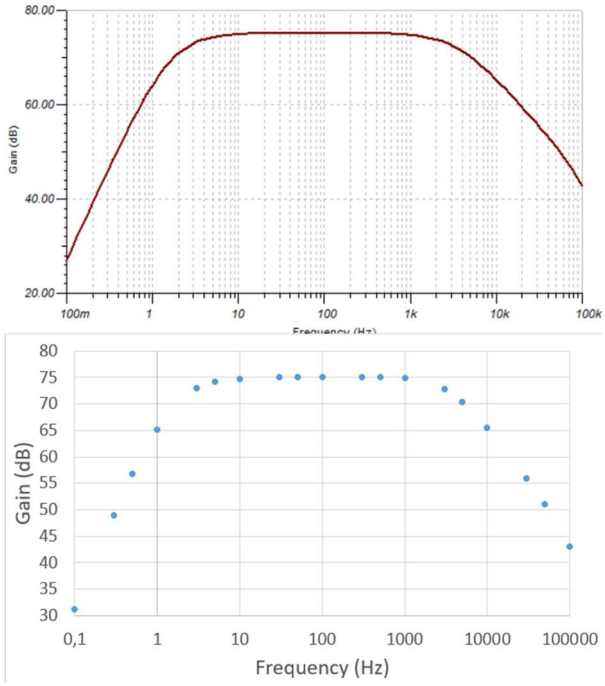


Fig. 3. Theoretical vs real bode diagram of the amplifier.

The second experiment consisted on the use of a MEA signal emulator (60MEA2100-SG, Multi-Channel Systems GmbH) which was used to simulate different signals representing several types of cellular activity depending on its configuration, such as hippocampal population and neuron spikes, heart ECG and retina ERG, all within realistic voltage ranges of those types of activities (varying between hundreds of microvolts to a few millivolts).
After feeding the amplifier with the simulated signals, as seen in the experimental setup in Fig. 4, results shown in Figs. 5-9 were recorded, where the recording capabilities of the amplifier is shown.

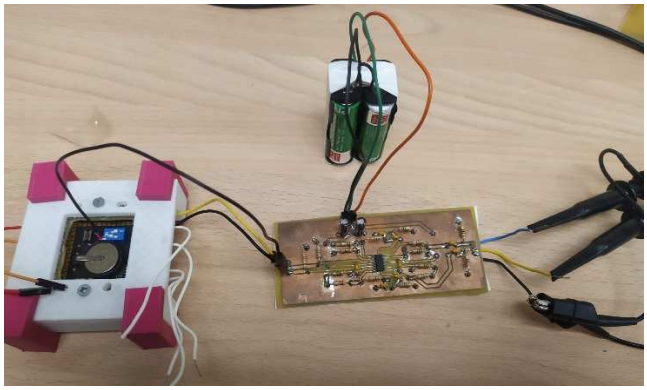


Fig. 4. Experimental amplifier setup

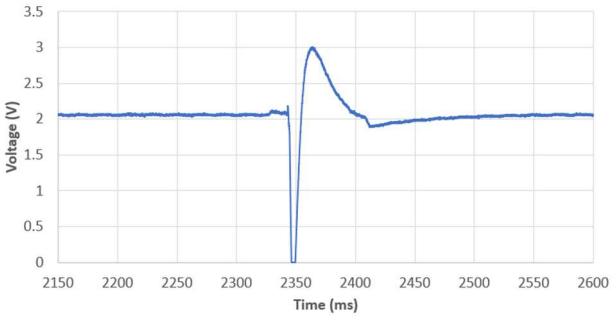


Fig. 5. Measured output from a simulator Hippocampal slice EPSP

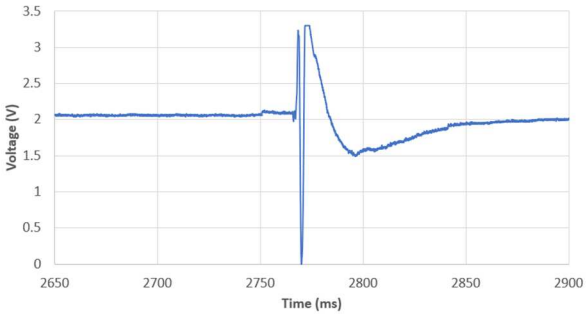


Fig. 6. Measured output from a simulator hippocampal slice Population spikes

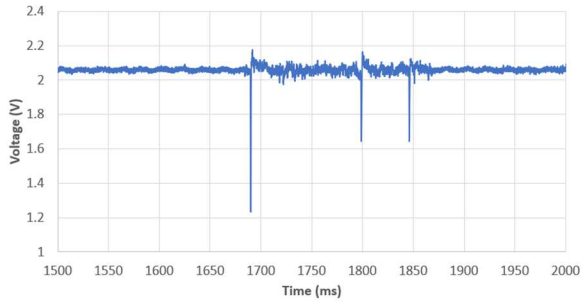


Fig. 7. Measured output from a simulator hippocampal slice neurons spikes

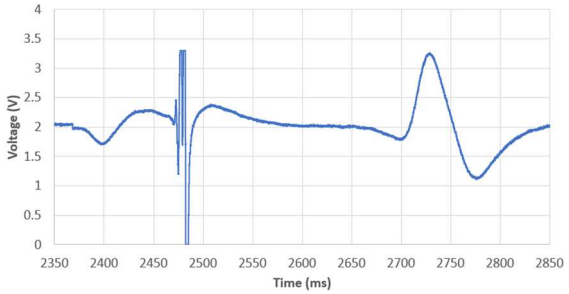


Fig. 8. Measured output from a simulator heart ECG ventricle

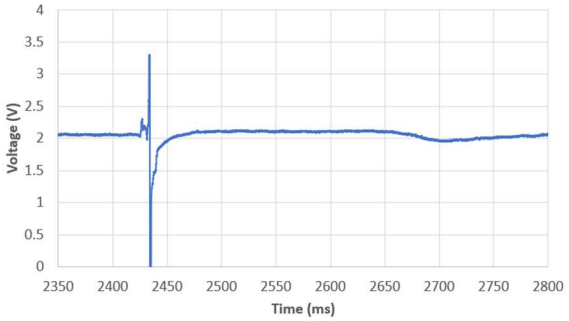


Fig. 9. Measured output from a simulator Cardiomyocytes Ventricle FP

After comparing the recorded waveforms of the amplifier shown in Figs. 5-9 with the expected output waveforms from the simulator, obtained from its datasheet [12] as shown in Table I, no noticeable distortion in the waveforms is observed. This shows that the amplifier is capable of amplifying the signals at its input without suppressing any frequency component important for the electrophysiological study, thus qualifying it for its use in the intended frequency range of 1 Hz to 3.3 kHz.

The last experiment consisted on the in vitro culture of real live cells in a standard 60 pads MEA (60MEA200/30iR-TI, Multi-Channel Systems GmbH).

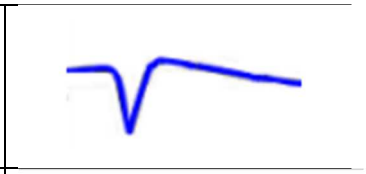
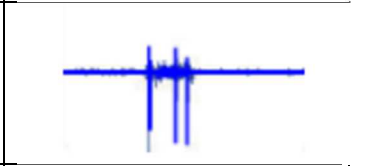
Hippocampal slice EPSP	
Hippocampal slice Population spikes	
Hippocampal neurons Spikes	
Heart ECG Ventricle	
Cardiomyocytes Ventricle FP	

TABLE I Output waveforms of the 60MEA2100-SG, Multi-Channel Systems GmbH simulator datasheet [12], shown for comparison with the experimental data presented later.

This MEA has 60 electrodes, each one of 30 micrometers width and spaced 300 micrometers from each other in an 8 by 8 configuration. The cells used were neuroblastoma cells sourced from ATCC, a biological materials resource and standards organization, with reference number SK-N-SH. After several in vitro experiments lasting anywhere from half an hour to a full hour, where the live cells cultured in the MEA were taken out of the cell incubator and tested in a lab at room temperature while slowly degrading, the following results were captured. The recorded signals have demonstrated similar morphology to some types of cellular electrical activity, which gives experimental validation of the working of the amplifier. A few samples of the captured signals are shown in Figs. 10-13:

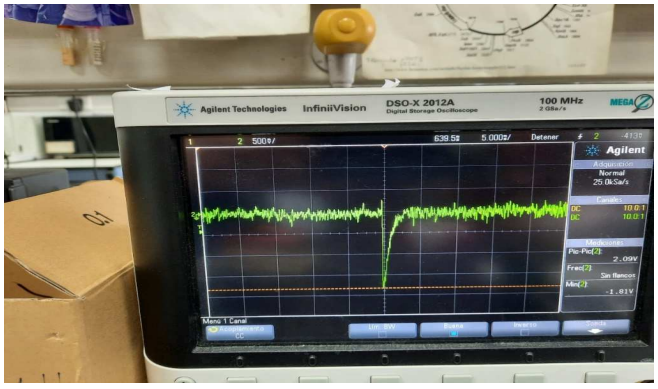


Fig. 10. experiment 1, cell activity

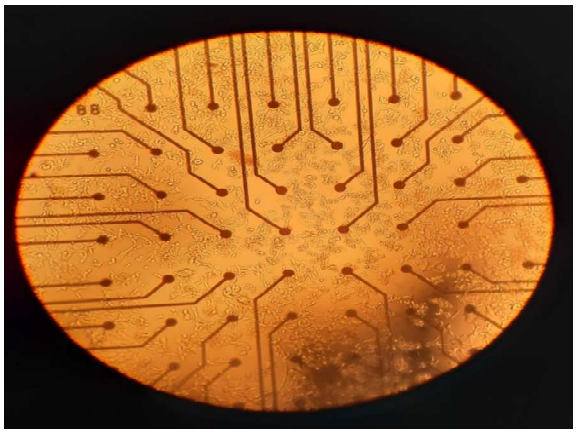


Fig. 11. experiment 1, cell culture

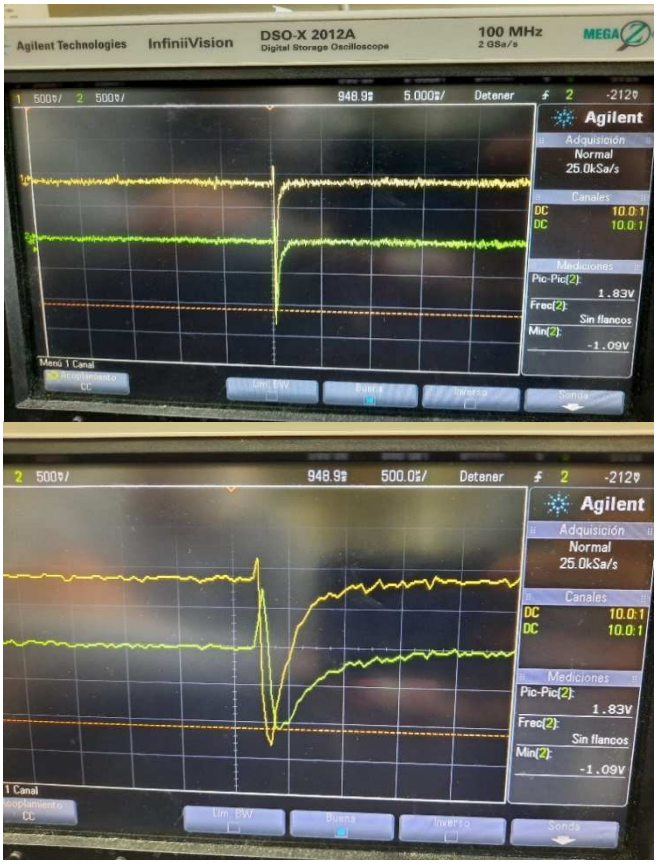


Fig. 12. experiment 2, cell activity

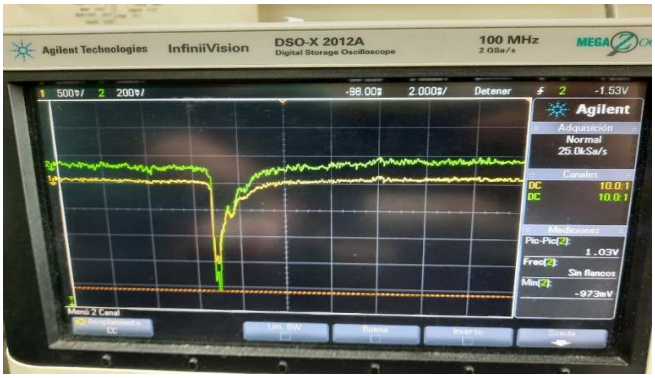


Fig. 13. experiment 3, cell activity

IV. Conclusions

In this article, the design process of an amplifier for planar titanium plated MEA signals, be these from cell cultures or tissue slices, is described, alongside with the justifications for most of the decisions taken along the design process. This amplifier has been fabricated, and extensive testing with both simulations and real in vitro cell experiments have been conducted in order to validate the amplifier design, and it has shown its capability to amplify actions potentials in real in vitro cell test.

The next steps will include the integration of 59 amplifier channels in a single board to simultaneously acquire all signals from a standard MEA, and the design of a robust solution for the recording and transmission of data from the amplifiers onto a computer that will store and analyze the information generated in the experiments.

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