# Experimental Measurements in the Acquisition of Biosignals from a Neuronal Cell Culture for an Exoprosthesis Command

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*This article presents experimental measurements performed in order to connect a neuronal cell culture to an exoprosthesis. The experiments focused on the biosignals' acquisition from the cell culture. A special gold-plated glass plate device was realized and several constructive variants were analyzed. A Olympus microscope with fluorescence and photo system was used. The acquisition of bio signals from the neuron culture is realized and described in the paper. The measurements were made in the sterile environment within the laboratory of Institute of Cellular Biology and Pathology. The measurements have been made for the pair of electrodes 1-1 at the edge of the glass plate.*

*Keywords: neuronal cell culture, bio signals, exoprosthesis, Clewin Mask Editor, neuronal command*

In vitro cultures of neuronal cells are worldwide used in order to better understand human nervous system and its biosignals. The culture of neuronal cells is a modern specific challenge, since mature neurons do not undergo cell division [1]. The information transmission through axons and dendrites represents the important feature of neuronal cells. A number of major factors are critical in the cultivation of these cells. The substrate on which the cells are seeded is one of the crucial one. The culture medium composition is another major factor regarding the cells growth and serum. The cell density has also a central importance as a general factor for most cells including neural ones. In order to achieve the best cell culture results, having the right components for complete neural cell culture medium is essential [2]. Growth and maintenance of neurons is an intrinsic operation.Cell survival rates are critical to the success and reliability of your neurobiology research. Many available products including protocols, to support modern research help in improving the reliability of scientific experiments by increasing the survival of neurons by more than 50% [2]. The term biosignal generally includes any type of signals acquired from living cells including both electrical and non-electrical signals (biomagnetic, biochemical, biomechanical, bioacustic, biooptical etc.) [3]. In this paper only electrical signals will be considered. Biosignals contain useful information to understand the physiological mechanisms [4]. They need to be analyzed in order to retrieve useful information. Basic methods of signals processing are: amplification, filtering, digitization and storage. Usually, in biosignals processing, a lot of different types of sensors are used in order to transform the bio signal into an electrical analog signal that can be measured by using a data acquisition system. This system converts the analog signal into a digital one in order to be stored. Specific electronic processing techniques are used to reduce noise and extract additional information from the digital signal [4]. In vitro cultures of neuronal cells, specific methods and protocols have been settled down for cell culture models developing [5]. The usage of in vitro based neuronal cultures has facilitated the analysis of human nervous system, regarding the effects of different electric, electromagnetic or biochemical stimuli. Due to the high complexity of neuronal networks, a detailed assessment of the culture's reaction remains still complicated [6]. Usually the investigations of neuronal networks are performed on a single cell level, so that changes in network activity or communication patterns can be correctly identified [6]. Trying to interface the electronic system with neuronal cells several approaches have been followed from using FETs (Field Effect Transistors) and metal mioelectrode arrays to detect extracellular neuronal signals [7]. The interface between the electrodes and neuronal cells must be stable and this is a critical point to achieve long-term signal recording stability [8]. The electrophysiological signals measured are in the range of microvolts, so these signals should be amplified by a factor of 1000 (or 30 dB) by a two-phase amplification system, with special techniques that avoid the amplification of noise [9, 10]. In this paper the useful signal has been captured using a glass substrate with gold electrodes patterned on top. The resulting analog signal is converted to digital, and the obtained value is processed with a microcontroller which will control the action of each finger from the prosthesis.

## Experimental part

### *Technological process for gold-plated glass plate manufacturing*

To obtain the set of masks, a dedicated writing equipment DWL66fs was used. The mask writing process with DWL 66fs is achieved through the help of a 405-nm Diode laser. DWL 66fs has 2 write heads of 2 and 10 mm, which ensure a maximum resolution of 0.6μm respectively 3 μm. The mask file format can be in any of the following

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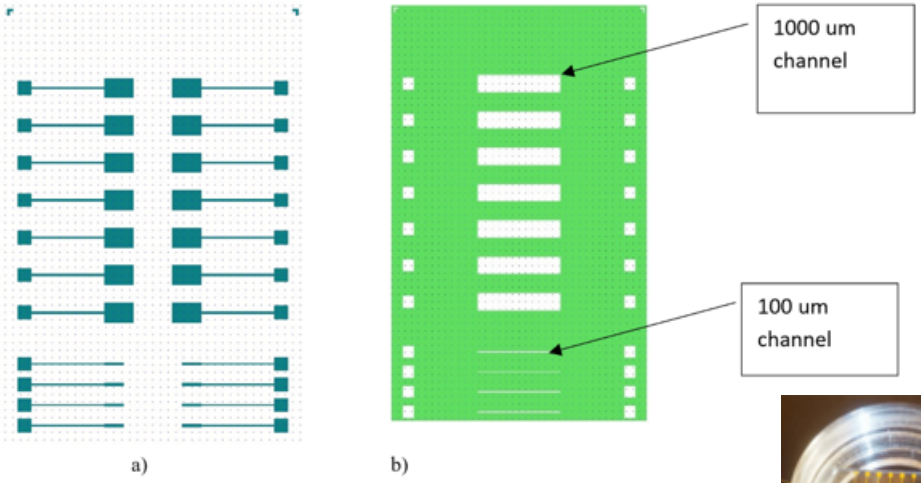


Fig. 1. The layout of the rectangular structure of gold tracks on the rectangular glass plate

extensions: DXF, HPGL, Gerber, GDSII or CIF. Writing facility: on mask (with dimensions ranging between 2.5" and 6") and on substrates (with the diameter of 3" or 4 "). It can be used for mask making or direct exposure on basically any flat material coated with photoresist. Numerous optional features increase the flexibility and make the system suitable for more applications. For titanium/ gold deposition a sputtering equipment has been used. It permits vacuum depositions of thin films of Ni and Au using DC sputtering system. The system is working al low temperatures, the metallic ions reaching the substrate at ~100 °C . The specific design for the glass wafer deposited with Ti/Au is shown in the figure 1. The layout was realized using the Clewin Mask Editor.

The technological processing steps are:

1) Substrate preparation3x3 inch glass substrate was used and the surface cleaning was carried out in Pyranhia solution, 5 min+ D.I water wash and rinsing 10 + 10 min;

2) Deposition of a Ti/Au layer with a thickness of 15nm/ 200 nm;

3) Photolithographic process (Mask 1 with the design from the fig. 1) , Ti/Au etching, and resist removal. After this step the electrodes are patterned as indicating in fig1. a) .

4) Once this stage has been completed, a layer of SU8 with a thickness of 100μm has been deposited on the glass surface to define the required channels. This last layer was configured with the help of the mask in form figure 1 b;

5) The last technological stage consisted in cutting the glass into chips, the chip size obtained after the separation being 15 x 23 $mm^2$. The SU8 channels thus obtained have the width dimensions of 100 and 1000 μm.

The second device layout for neuronal cells monitoring and measurement is presented in figure2. The technological steps are identical with those presented above for device 1 but the masks geometries are different.

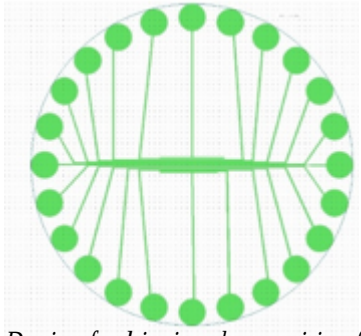

Fig. 2. The layout of the circular structure of gold tracks on the circular glass plate

*Device for biosignals acquiring from neural cell culture*

In order to acquire the signals from cell culture, two similar devices have been made as the general structure, but different in terms of electrode configuration. In the first

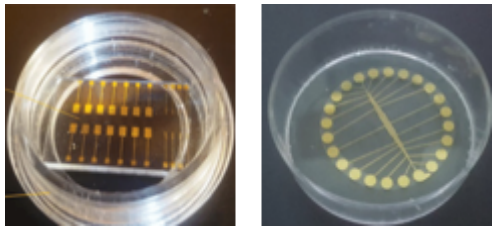

Fig. 3. The two rectangular glass sheets (a) and circular shape (b)

device (fig. 3.a) the acquisition electrodes were placed in a rectangular shape on the rectangular glass plate. In the second device the electrodes were placed in a circular shape on the circular-shaped glass plate (fig. 3.b).

The device's components for the acquisition of signals taken from the neuron culture are shown in the Figure 4. The upper plate (1) is fastened above the bottom plate (5) in which the culture bowl (8) was positioned using four screws (9) so that the contracted pins (2) overlap exactly the ends of the printed gold paths on the glass plate (4); the four screws (9) provide a pushing force of about 15 N, required to produce a 30 milliohms contact between each contractile pin and the corresponding path on the glass plate;

To avoid contamination of the environment in which the neurons develop, chemically inert (biocompatible) materials were used:

- The contractile pins have a bronze-phosphorus body; the copper rod is made of copper-beryllium, fully covered with gold;
- The paths on the glass plate are also made of gold;
- The culture vessel is made of chemically inert plastic, having the lower part made of 0.2 mm glass, which allows the visualization of the neuron fixation on the glass plate by microscope;
- The two glass-tiles have been coated with a protective lacquer (PLASTIK 70) to prevent moisture absorption from the environment.
- Sterilization of the acquisition devices was carried out in three stages: ionized water, ethanol and UV.

For signal acquisition from the plate on which the neurons are attached the following hypothesis was considered: at least one neuron is fixed between two gold electrodes from the glass plate. In this situation, the signals from the fixed neuron can be acquired when the cell culture is stimulated by a chemical method using caffeine. The functional scheme will be that of figure 6. For this situation, the neuron acquired signals will be processed to control the prosthesis mobile elements movement.

For a supplementary safety, the visual detection of the electrodes on which the neuron was fixed using a Olympus microscope with fluorescence and photo system was used. With the microscope image help, the user manually selected the two electrodes from which the useful signal will was collected (fig. 7).

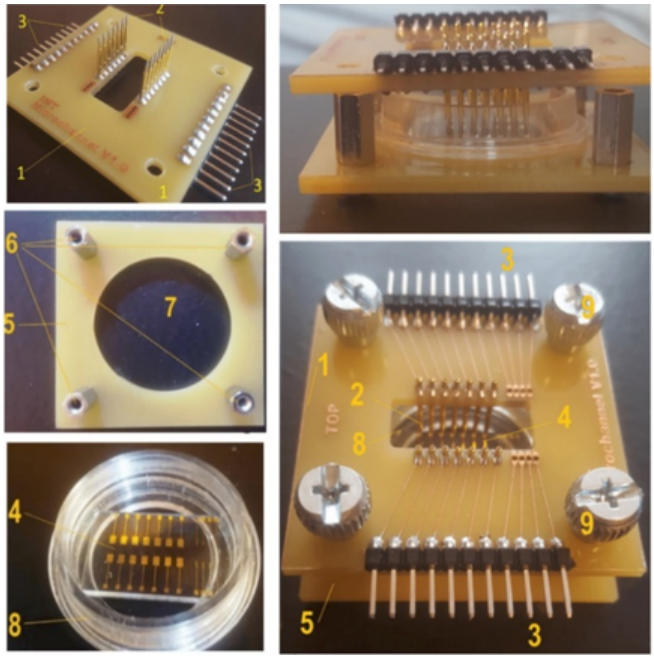

Fig. 4 The first device's parts for biosignals acquisition from cell cultures

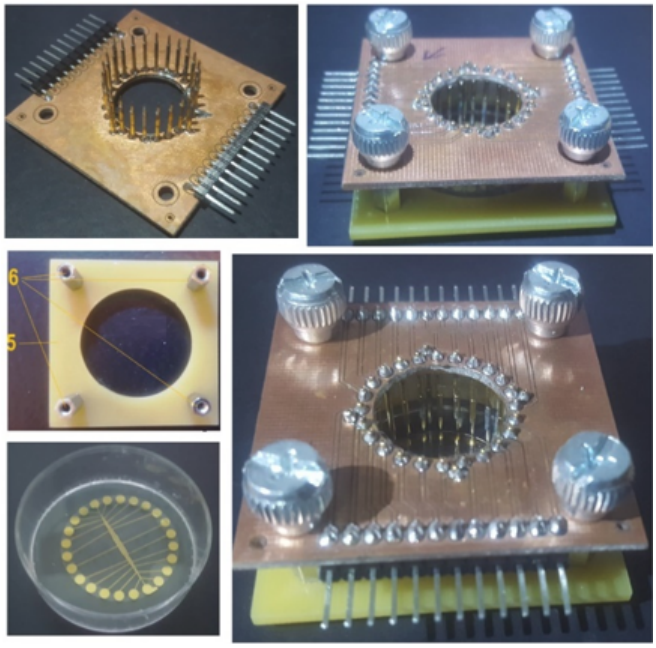

Fig. 5 The second device's parts for biosignals acquisition from cell cultures

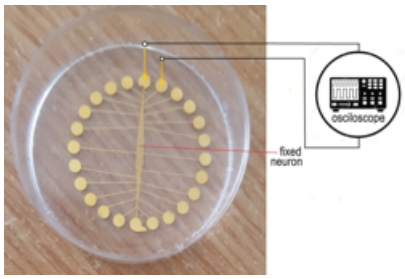


Fig. 6. The functional scheme of the neuron culture signal acquisition system and the oscilloscope waveform

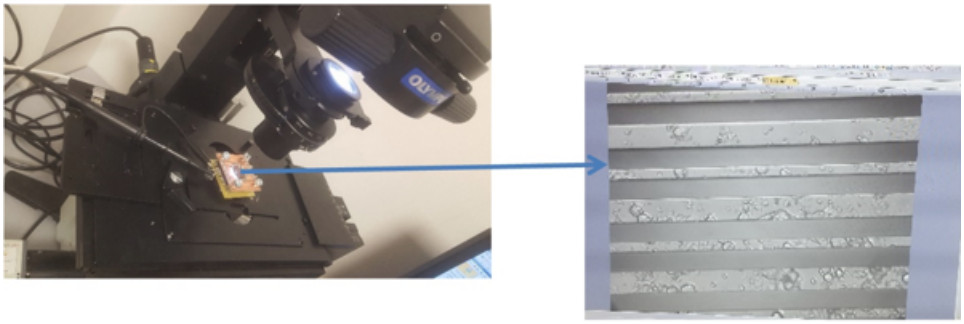

Fig. 7. Microscope view of neuron cell culture on the electrode plate

## Results and discussions

In the performed experiments a neuron culture of spinal ganglia has been used. It was provided by the applicants from the University of Bucharest, Faculty of Biology. The culture of neurons has been prepared 24 h before measurements. The measurements were made in the sterile environment within the laboratory of the Faculty of Biology.

Two experiments were performed one for each device. The experiment with the circular glass plate gave better results. The measurements were initially carried out without shielding and in the acquired signals was present the network noise (50Hz) of about 18 mVvv (vv = top peak) (fig. 8). Then the measurements were resumed in a Faraday cage connected to the inland belt of the building and the noise signal has been reduced 3 times (at about

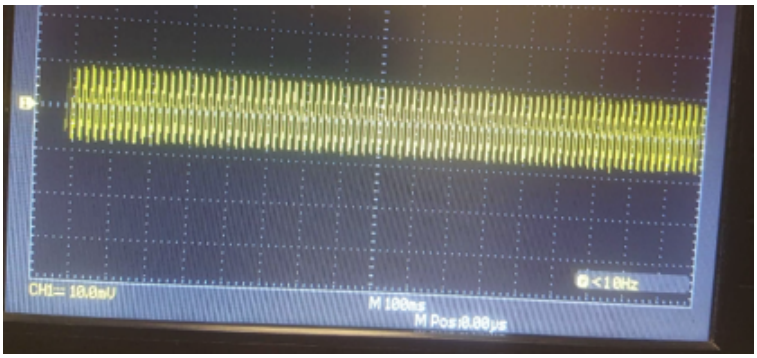

Fig. 8. Network noise (50Hz) of about 18 mVpp (pp = peak to peak )

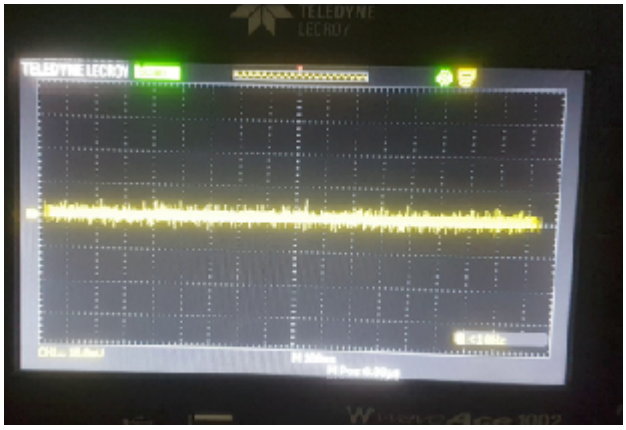

Fig. 9. Measurements in a Faraday cage linked to the building's belt: reducing the network noise 3 times (at about 5mVpp)

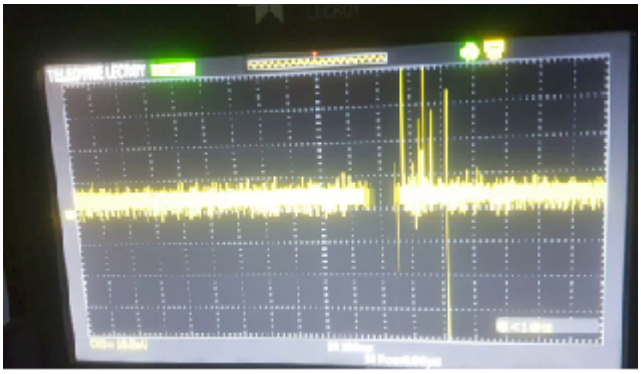

Fig. 10. Acquisition from the cell culture of a pulse train of amplitude 20-30mVpp and duration of 300 ms

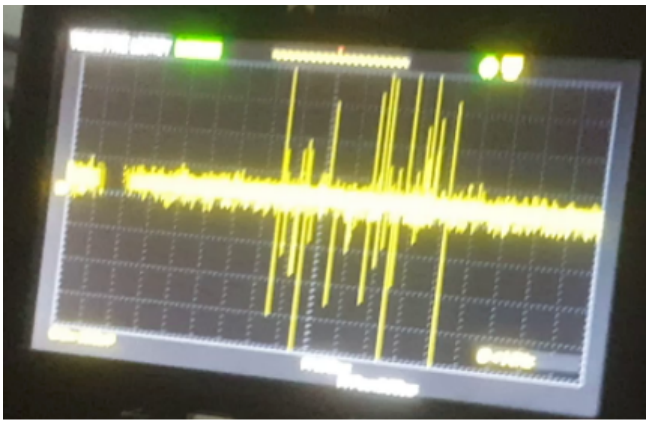

Fig. 11 Acquisition from the cell culture of a pulse train of amplitude 20-30mVpp and duration of 600 ms

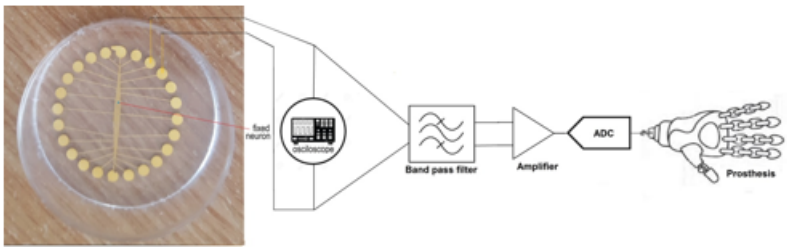


Fig 12. The functional scheme of the system for the acquisition of signals from the culture of neurons and the command of prosthesis

5mVvv) (fig. 9). A drop of caffeine was then applied to the solution containing the cell culture. The caffeine solution produced excitement of all neurons, including those fixed on electrodes, and a pulse train 20-30mVvv (fig. 10) was acquired from the neurons fixed on the gold electrodes approximately 50 seconds after applying the caffeine solution. The duration of the pulse train varied between 300 and 600 ms (fig. 11).

The recorded signals have a wide range of waveforms and amplitudes. The useful signal captured from the two electrodes had amplitude of amplitude 20-30mVpp and duration of about 300 - 600 ms; it was filtered, eliminating the high frequency components and then amplified about 150 times. The resulting analog signal was converted to digital, using a 12-bit analog-to-digital converter. The value obtained was processed with a ATMEL ATmega microcontroller, which controls the action of a finger from a prosthesis. The signal from the cell culture was used to control the finger of a prosthesis [11-12] that allows finger movement command with external signals via USB port (fig 12).

## Conclusions

In this paper a neuronal culture (spinal ganglia) in *vitro* has been analysed as a system special designed to study and demonstrate a possible connection of a human neuronal network with an exoprosthesis. Two devices with different designs (rectangular and circular) were fabricated for the acquisition of signals from the neural culture and several experiments were carried out. Each device contained a glass plate on which several gold electrodes were fixed. Spinal lymph node culture was prepared for fixation, incubation, and growth 24 h before the measurements. Only the fixed neurons in the channels from the glass plate were connected using a pair of electrodes at the edge of the glass plate. Caffeine supplementation gave a significant stimulation of neurons and permit acquisition signals from neurons fixed in channels on glass wafer. The best results were obtained in the experiments performed with the circular glass plate device. From the neuronal culture, signals with amplitude of 20-30mVpp and duration of 300 - 600 ms were acquired. These signals were processed (filtered, amplified and converted into digital signals) and used to control the movement of a finger of the prosthesis. The results obtained in these experiments have shown that signals acquired from neurons can be used for an exoprosthesis command. Signals acquired from the peripheral nervous system of the patient's stump have far better characteristics for a exoprosthesis command, compared to myoelectric signals (purchased from the muscles of the patient's forearm). The obtained results recommend further research into the acquisition of signals from the peripheral nervous system from a patient's amputation stump to efficiently control an exoprosthesis.

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## References

1. JENNIFER GORDON,SHOHREH AMINI, MARTYN K. WHITE, General overview of neuronal cell culture, National Center for Biotechnology Information, U.S. National Library of Medicine, USA, Methods Mol Biol. 2013; 1078: 1-8, doi:10.1007/978-1-62703-640-5_1. 2014; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052554/

2.*** Thermo Fisher Scientific, https://www.thermofisher.com/ro/en/home/life-science/cell-culture/primary-cell-culture/neuronal-cell-culture/neural-cells.html

3.***Biosignal Processing, Kyung Hee University, http://web.khu.ac.kr/~tskim/BME101_08_1_Biosignal%20Processing%20(I).pdf

4.SHOHREH AMINI, MARTYN K. White, Neuronal Cell Culture, Methods and Protocols, ISBN 978-1-62703-640-5, Springer Publishing House, 2013, https://www.springer.com/us/book/9781627036399

5.*** Aschaffenburg University of Applied Sciences , BioMEMS Lab, Neuro-Biosignal Processing,https://www.h-ab.de/eng/about-aschaffenburg-university-of-applied-sciences/organisation/labor/biomems/research/neuro-biosignal-processing/

6.ANDREAS OFFENHAUSER, ROSS RINALDI, Nanobioelectronics - for Electronics, Biology, and Medicine, Springer Publishing House, ISBN 978-0-387-09458-8, 2009

7.TAO SUN, WEI MONG TSANG, WOO-TAE PARK, KANGJIAN CHENG, SRINIVAS MERUGU, Modeling in vitro neural electrode interface in neural cell culture medium, Microsystem Technologies,Volume 21, Issue 8, pp 1739–1747, 2015,https://link.springer.com/article/10.1007/s00542-014-2292-7,

8.FRANCISCO FAMBRINI, MARCO ANTONIO BARRETO, JOSE HIROKI SAITO, Low noise microelectrode array signal headstage pre-amplifier for in-vitro neuron culture, 2014 IEEE Conference on Computational Intelligence in Bioinformatics and Computational Biology, https://ieeexplore.ieee.org/document/6845522/

9. CARMEN MOLDOVAN, RODICA IOSUB, CECILIA CODREANU, BOGDAN FIRTAT, DANIEL NECULA, COSTIN BRASOVEANU AND ION STAN, Miniaturized Integrated Platform for Electrical and Optical Monitoring of Cell Cultures, Sensors **2012**, 12, 11372-11390; doi:10.3390/s120811372

10. MONICA DASCALU, Cellular Automata Hardware Implementations - an Overview, Romanian Journal for Information Science and Technology, Volume 19, Number 4, 2016, 360–368pp.

11. P. L. MILEA, M. DASCALU, E. FRANI, A. BARBILIAN, I. C. STOICA, Tactile Feedback Experiments for Forearm Prosthesis with Myoelectric Control, pp. 101-114, Romanian Journal for Information Science and Technology, Volume 20, Number 2, 2017;

12. P.L. MILEA, M DASCALU, C.O. OPRIS E FRANTI, M. DUMITRACHE, C I STOICA, Using pressure sensors for motion detection and actuation of remote manipulation, Romanian Journal for Information Science and Technology, Volume 19, Number 4, 2016, p:321–330.

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