Implantable optical-electrode device for stimulation of spinal motoneurons

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Abstract. Recent years, optogenetic method of scientific research has proved its effectiveness in the nerve cell stimulation tasks. In our article we demonstrate an implanted device for the spinal optogenetic motoneurons activation. This work is carried out in the Laboratory of Molecular Neurodegeneration of the Peter the Great St. Petersburg Polytechnic University, together with Nano and Microsystem Technology Laboratory. The work of the developed device is based on the principle of combining fiber optic light stimulation of genetically modified cells with the microelectrode multichannel recording of neurons biopotentials. The paper presents a part of the electrode implant manufacturing technique, combined with the optical waveguide of ThorLabs (USA).

1. Introduction

It is proved that each separate group of brain neurons is responsible for performing a specific elementary function. And it is the interplay between these systemic units that ensures the whole neural network operation. Until recently obtaining of experimental data was mostly limited to the studying of the consequences resulted from damages to the certain parts of brain or to the record of the brain activity during animal's execution of stereotype tasks [1].

In terms of the therapeutic effect, researchers used electrical stimulation and pharmacological products most frequently. In order to activate different parts of brain they implanted electrodes into them. In this case, however, electrical current affects almost all the undifferentiated neuron groups (electrode stimulates all neural tissues) and, as a result, it was very difficult to localize a function's generator. When they used pharmacological products that could selectively inhibit nerve cells of a specific group, the effect of the chemical substance determined the time delay compared with the natural neural stimulation [2].

2. Optogenetic method

The application of a fundamentally new approach to the studying of brain neurons processing is optogenetics. An important advantage of the optogenetic research method is selectivity in terms of affecting the specific part of brain or the neuron group. Milliseconds matching data allow researchers to carry out experiments with the speed of the living cells biological response when determining the significance of research models specific actions in neurons. [2]

For combined optogenetic experiments with the use of electrophysiological stimulation of freely behaving animals, we use implantable cannulas that unite optical fiber and implanted electrode. This configuration offers real-time and accurate probe of the influencing organs to the experiment area.

3. Optical-electrode interface

Brain function studying requires neuron interface that could record parameters and stimulate brain with high time-space accuracy. Most researchers who use optogenetic method in laboratory conditions on *in vivo* animals now use optical fiber that is sent through the implantable cannula [3]. Parameters of the pulses sequence and their generation is controlled by computer graphical interface or manual switch of modes.

Programmed LED control drivers ensure the setting of direct current values for one or several separate LEDs or a cluster that consists of several diodes united in one output fiber. Each channel is controlled autonomously (manually in modes of CW, external TTL or analog modulation types) or by software installed on computer.

At the department of Medical Physics and in the Laboratory of Molecular Neurodegeneration we are working on the development and testing of implantable device for monitoring of brain neurons physiological parameters (action potential).

Together with "Nano and Microsystem Technology" research laboratory we are developing a combined optical-electrode device that allows you to carry out combined research with the use of intravital microelectrode stimulation and optogenetic activation of genetically differentiated neurons (figure 1).

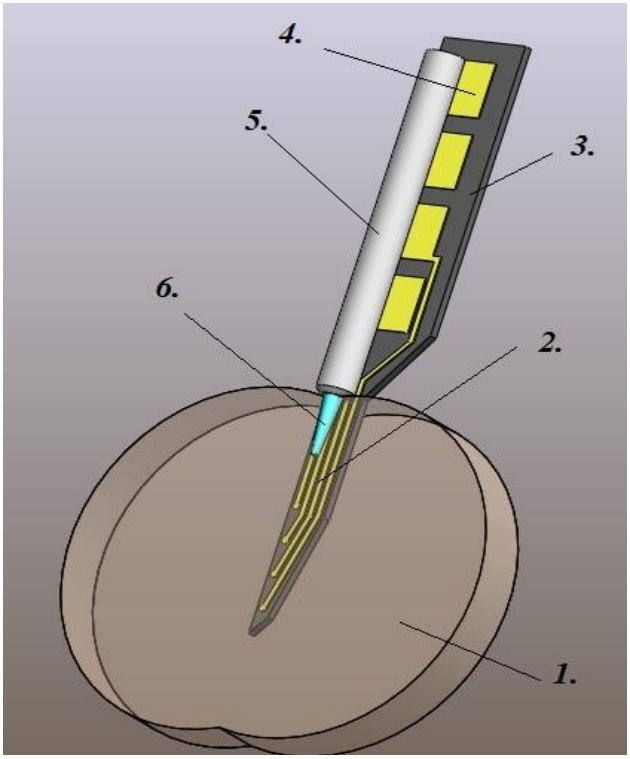

Figure 1. Implantable device layout: 1 – biopsy sample containing modified photosensitive motoneurons of the spinal cord; 2 – Submersible microneedles tip with electrodes outputs; 3 – silicon substrate of the implant; 4 – contact pad of output cable; 5 – light guide conductor; 6 – light guide tip.

4. Microneedle manufacturing technology

The first experiments on registration electrical activity from cells in vitro with the electrodes of 30-element microelectrode arrays were performed over 40 years ago. The multi-electrode array was fabricated by etching thin metal films deposited on glass cover slips; the fabrication employs techniques developed by the microelectronics industry [5].

To date, a plenty of probes have been used in neuroscience research based on multi-electrode arrays fabricated by Micro Electro Mechanical Systems (MEMS) [6].

The following section describes the development of base silicon chip design and technology containing needles with metal electrodes and contact pads for acquisition system.

Many research groups investigating neural probe technologies are faced with different challenges including non-standard and unconventional fabrication processes leading to low yield and high cost, lack of on-site and monolithic Integrated Circuit (IC) integration leading to high noise and reduced sensitivity, shorter sized probes mainly limited by fabrication technology, low design flexibility, and limited selection of materials having the mechanical properties that fulfill both the implantation application requirements and being compatible with standard micro fabrication processing [6].

Preliminary design is based on the composite scheme, combining micro fabricated silicon chip with microelectrode array and optical fiber. Chip design is shown in figure 2.

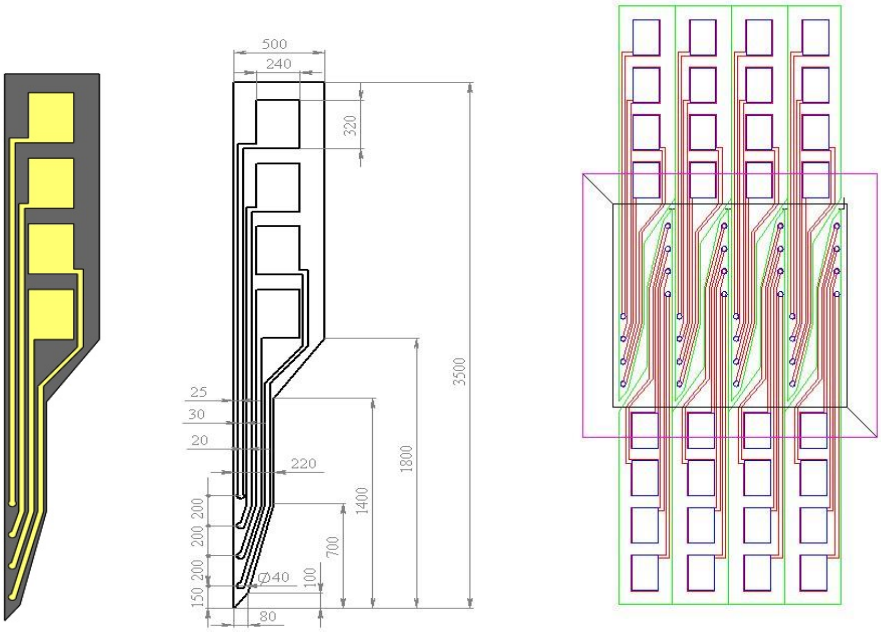

Figure 2. The appearance of a single needle chip, mutual arrangement of needles on photo mask.

Dimensions – 3500x500x400 μm, electrode diameter – 40 μm, number of electrodes – 4.

A given shape needles are made by step-by-step etching a standard silicon wafer in wet etching and dry plasma etching by Bosch-process. For pattern of metallization, dielectric isolation and contact pads we are used standard microelectronic photolithography and vacuum deposition techniques.

The standard silicon wafer (100) with dual side alignment signs is processed by wet etching at a back side depth of 350 μm under nitride mask. After metal film deposition, deposition of dielectric isolation and autopsy contact pads, perform deep silicon etching by Bosch-process. As result wafer is divided into individual chips - needles.

Silicon chip technological sequence schematically shown in figure 3.

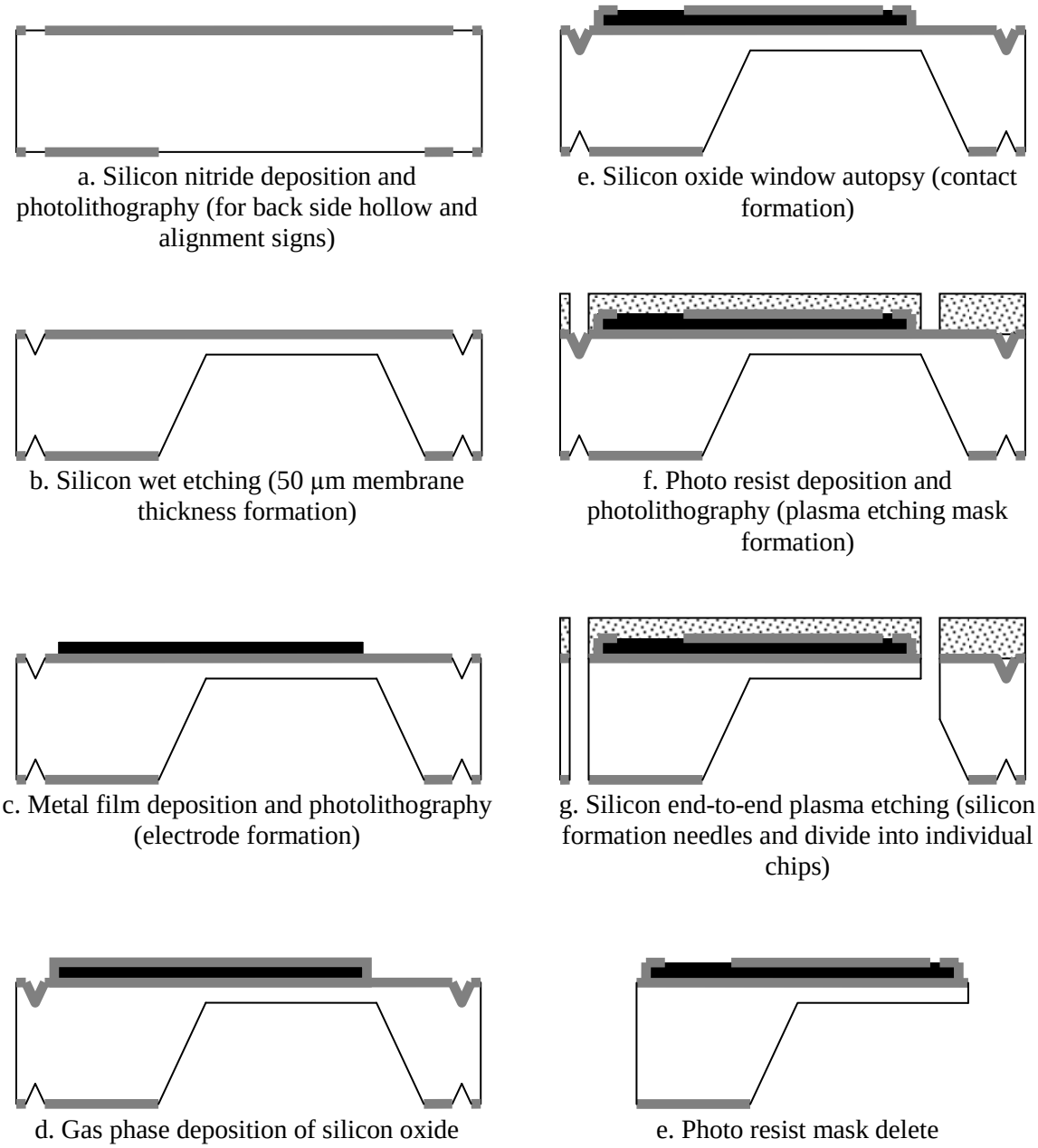

Figure 3. A schematic representation of the process of silicon chip technological sequence

The next step of experimental studies will be the production of microelectrode implants consisting of the micro needles and fiber group represented in figure 1. It is located in the central part of the device to obtain a three-dimensional data array of the distribution of neurons excitation at different distances from the radiation source (figure 4).

An implant consists of the coaxial conical optical waveguide (optrode) integrated inside the implantable electrode array (multi-electrode array-MEA) for recording the experimental data.

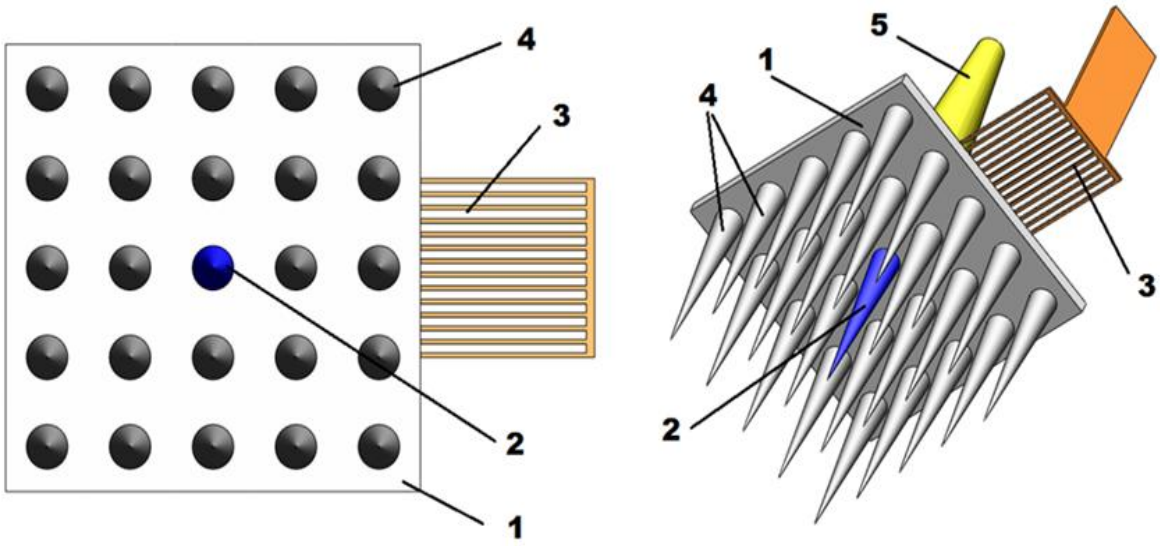

Figure 4. Schematic representation of an implantable optical-electronic array: 1 – the body of the array; 2 – integrated optrode; 3 – multichannel electrical data recording; 4 – electrode needle; 5 – optogenetic control signal

Optrode allows to record electrical activity during optogenetic experiments. This combination of several microelectrodes allows to record the activity of several neurons in light affected areas. It minimizes the effects of light diffusion inside the tissue and the mismatch of positions of the light source and the detector that records neurons excitation / inhibition parameters. [4]

5. Future plans and conclusions

Neurointerface is used in problems of activation and one-time registration of neuronal activity and is constantly being improved. Our future plans include using the device in long-term experiments on the spinal cord motor neurons stimulation using optogenetic techniques. The use of the implant is planned in slices and live animals like AD models. Simultaneously, the recording system, the filtering and processing of data received from the device are improved.

Acknowledgments

The authors express their gratitude to I.B. Bezprozvanny, the head of the Laboratory of Molecular Neurodegeneration, doctor of biological sciences and Carl J. and Hortense M. Thomsen Chair in Alzheimer's Disease Research, and to the whole team of the laboratory for consultations and assistance in research.

The part of the study dedicated to staging and testing optogenetic methods is supported by the Russian Scientific Fund Grant no. 14-25-0024.

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