

Vertical nanoelectrode system for potential measurement of living cells

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Abstract

Nowadays the measurement of electrical potential in living cells is a very trendy analysis in the sphere of biotechnology. The cells communicate with each other with the help of electric signals. Additionally based on certain changes in the electrical potential it is possible to observe the reactions of living cells in their life cycles or reactions due to various external influences. The system of measurement must be capable to observe extracellular potential continuously without causing trauma or damage in living cells. Taking into account the above-mentioned requirements a novel vertical nanoelectrode system for a long term cell analysis in water based medium was designed and realized [1, 2]

The two-electrode system operates on the basis of impedance sensors. Dimensions of electrodes require a combination of micro and nanotechnology techniques for fabrication. For the base of chip a silicon wafer coated with thermal silicon oxide with a thickness 500 nm was used. The first part of realization was the preparation of electrodes for measurement device and the mark for lithography. The electrodes and the mark were made of chromium nickel adhesion layer and PVD (Physical Vapor Deposition) gold layer. The motive was realized with standard UV lithography processes with AZ 5214E resist, and etched with gold etchant standard (Sigma Aldrich) and with NiCr etchant on base of Ammonium Cerium (IV) Nitrate $((\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6)$. After the wet chemical metal etching, the rest of the photoresist mask was stripped away with acetone and isopropanol.

The second part of realization was the preparation of vertical nanoelectrodes. The contact between electrodes for measurement device and vertical nanoelectrodes is ensured by two horizontal submicron electrodes with width of 150 nm. To achieve this line resolution at gold structures it is necessary to use accurate etching technique. The most appropriate alternative in this case was etching with focused ion beam (FIB). [3] The submicron electrodes and the mark for electron beam lithography (EBL) were etched into gold and NiCr layer with FIB. After etching processes the sample was prepared for fabrication of vertical nanoelectrodes in two steps. The first step was coating on the surface of sample PMMA resist, the second step was creating two nano holes into the polymer layer with EBL. The holes must be located over the edge of the horizontal submicron electrodes. The ideal size of holes is 300 nm for depth and 100 nm for diameter. These sizes are based on requirements of measurement method. The measured cell must be electrically isolated from conductive gold layer, and must be in contact with gold nanoelectrodes. The appropriate thickness of PMMA resist for these applications is 300 nm or more.

The last part of realization was filling the nano holes with gold. The most accurate method for filling nano holes is voltage controlled pulsed electrochemical deposition of gold. Electrolyte on base of gold potassium cyanide was used for deposition. After the deposition process it was necessary to clean the sample with water and isopropanol. The PMMA layer must not be removed, because it serves as an isolator layer. The structure of prepared system is visible on SEM images (Fig. 1, 2).

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Figures

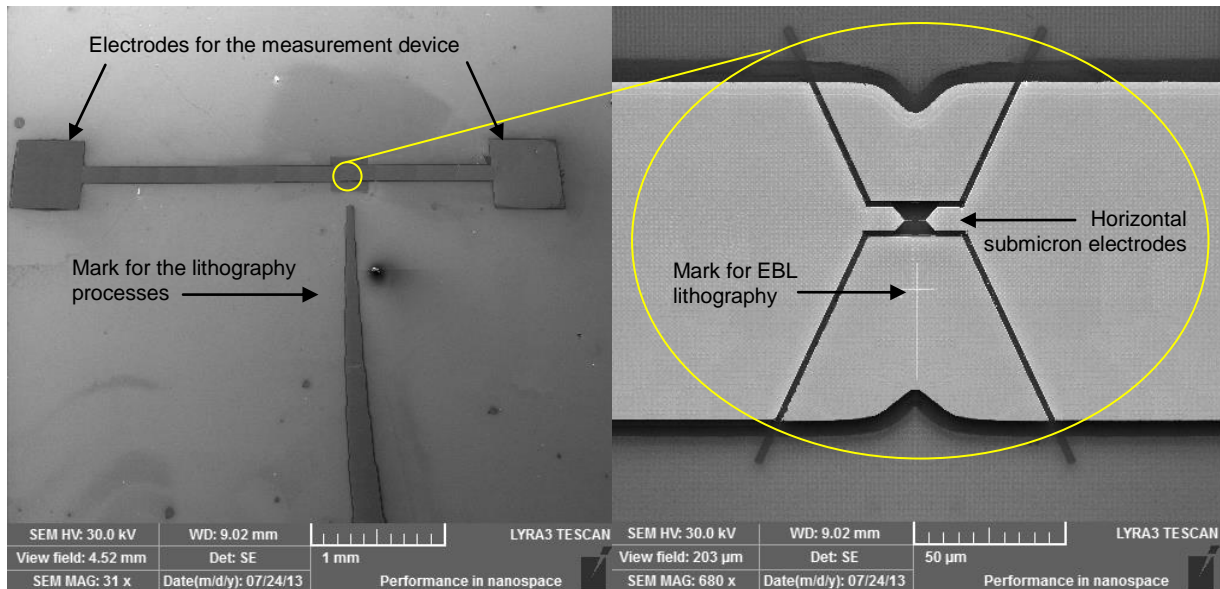


Fig. 1. SEM image of electrode system. (left) SEM macro image of horizontal submicron electrodes. (right)

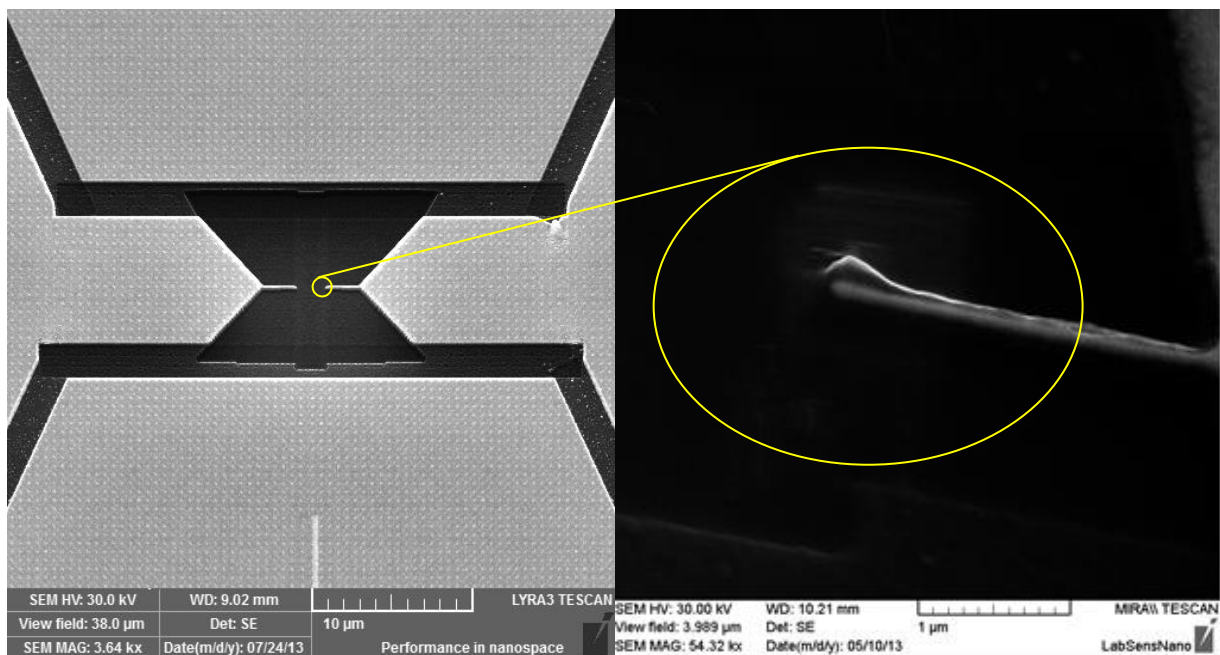


Fig. 2. Details of submicron electrodes and about a place, where vertical nano electrodes are deposited. (left) Vertical nano electrode on the edge of the horizontal submicron electrode. (right)