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Silicon Nanowire as Virus Sensor in a Total Analysis System

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Abstract

Silicon nanowires are very promising candidates for the sensitive detection of viruses or even early detection of cancer. Due to their small dimensions the attachment of even one particle on their surface leads to detectable changes in their conductivity. In this paper we describe the development (fabrication and testing) of a silicon nanowire biosensor equipped with microfludic channels and automatized data aquisition for the detection of antibodies against a small virus (Aleutian Disease Virus) causing plasmacytosis on mink and ferrets.

Keywords: Silicon nanowires; measurement automation, microfabrication, plasmacytosis

1. Introduction

In recent years vast progress has been achieved in producing ultra sensitive nanosensors with unique properties for biochemical analysis, highly sensitive and specific detection of biological molecules open for very early diagnostics, disease progress evaluation, virus screening and personalised medication. The silicon nanowire (SiNW) used as a biological Field Effect Transistor (BIOFET) [1] has proven to be a very good candidate for extremely sensitive detection down to a single molecule [2,3]. Although the field is still being established, several published papers have shown the potential of the system. The first step is to make the surface prone to detect the desired biological entity, e.g virus antibodies. This is carried out by fuctionalising the surface of the SiNW for specific recognition. The biotin-streptavidin recognition system is often used as a prototype, as shown first in [1] and later in [4-7]. The first virus detection scheme was illustrated by Patolsky et al in 2004 [8]. A few other publications exist using different functionalisation schemes for detecting various viruses [9-12]. The NW–BioFET has also been used as a drug screening device [13] and in genetic testing [14-17].

Using SiNW as the sensing element presents some challenges, i.e. getting the analyte to the sensor in

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a controlled fashion and enabling real time measurements. The best way to achieve environmental control is using a microfluidic approach. Here silicon nanowire sensors will be presented in combination with a fully automated custom built microfluidic platform, controlling the chemical handling and data analysis.

One of the issues minimizing the commercial potential of SiNW based biosensors is the large fabrication cost per chip since the fabrication process requires the use of e-beam lithography [18]. In this work we are demonstrating that one dimensional (1D) SiNW, also known as nanoribbons, can be used in the same way as SiNW but at a much reduced cost per chip. These 1D SiNW are fabricated using standard microfabrication processes, making batch processing feasible. Electrical connections to the external equipment are achieved through Zif sockets. The fabrication process, the custom built microfluidic set-up and the preliminary results towards the detection of antibodies for the smallest existing virus type, i.e. the parvovirus (ADV) leading to plasmacytosis in mink and ferrets, will be discussed in this article.

2. Fabrication

Silicon on Insulator wafers are used to fabricate the SiNW as seen in fig 1. The wafers are dipped in an HF bath for 20 seconds to remove the native oxide on the silicon. Then a photolithographic step defines the pattern of the silicon nanowires, and a reactive ion etch transfers the pattern to the wafers, fig 1.2. Then another lithographic step defines the highly doped regions on the nanowires, and the doping is performed using ion implantation, the red regions in fig 1.3. The low doped region on the nanowires is fabricated in a Boron pre deposition step, the pale regions in fig 1.3. The metal contacts are then defined using metal deposition and a lift-off process, fig 1.4. And finally the microfluidic channels are defined in SU-8 using a standard photolithographic step, fig 1.5. The wafers are then diced and ready to use.

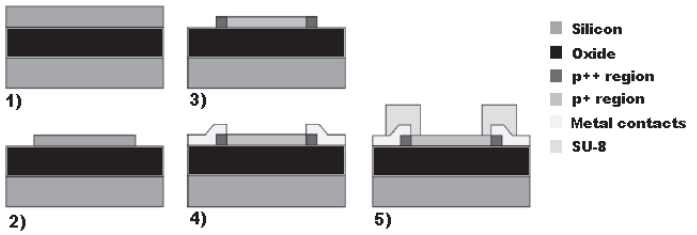


Fig. 1. The SiNW process sequence. 1) SOI wafers were used to define the height of the NWs 2) A standard UV lithography process and reactive ion etching is used to define the SiNWs on the wafers 3) Ion implantation and Boron predeposition is used to dope the NWs 4) Metal deposition and lift-off defines the metal contacts on the chips 5) The last step is microchannels fabricated in SU-8.

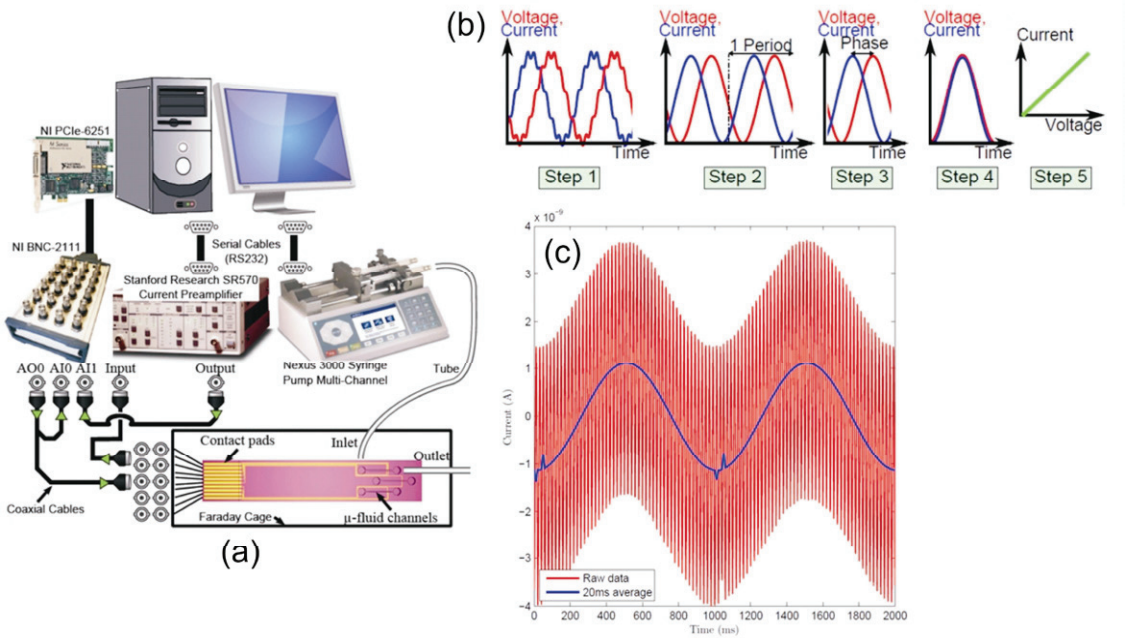
3. Measurement system

The sensitivity of the measurements depends on how well the NW is designed but also on the precision of the external electrical measurement system. Performing accurate measurements involves using the right principles, reducing noise sources, choosing suitable filters, and doing suitable data analysis.

The aim of the system is to perform measurements of the NW resistance over time in the 100 kΩ to 1 GΩ range. This requires a remote controlled current preamplifier. Due to the large upper value of the resistance range the measured current signal is small, which introduces parasitic capacitances, large 50 Hz and 100 Hz noise, as well as amplifier self oscillations. The complete measurement system is shown in fig. 2a. A Labview program was developed to control all the equipment and perform real time signal processing to remove the noise and improve the signal. The data is processed in 5 steps (fig. 2b). An example of a raw signal and the signal after applying a 20 ms moving average filter is shown in fig. 2c.

The Labview program also controls the syringe pump that feeds the chip with the various chemicals required for the experiment. In refill mode the chemicals are loaded into the syringe and separated by air

gaps. In infuse mode the chemicals are fed to the chip in the reverse order they were loaded. The user has complete control over the entire program through a user-friendly interface and can decide the order with which the operations will take place. The user also decides the filter to be used. The frequency response



of the three available digital filters is shown in fig. 3a.

Fig. 2. (a) The entire measurement system. The PC is connected to a LabVIEW device, a current preamplifier and a syringe pump. IV- and impedance measurements can be performed on the SiNW on the chip, while controlling which chemical the NWs are in contact with. (b) The resistance measurement principle. Step 1: the applied voltage and the current through the chip are measured. Phase differences between these signals are due to parasitic capacitances. Step 2: noise is reduced by controlling the analogue low-pass filter (LPF) in the current-to-voltage amplifier and by using digital FIR-filters. The FIR filters remove the 50 Hz and 100 Hz noise, which are the most prominent in the signal (fig. 3a), while the analogue LPF removes the self oscillations. Step 3: 1 period of data is cut out for analysis and the phases of the voltage and current are determined by fast Fourier transform (FFT). Step 4: these phases are used in order to align the current data and voltage data. Step 5: the aligned data are used to plot current vs. voltage and calculate the SiNW resistance. (c) Two periods of the measured current vs. time. The red curve shows the main (and noisy) 1 Hz signal. The noise is effectively removed by the 20 ms moving average filter (blue curve).

4. Results and conclusion

To test the ability of the SiNW to function as a biosensor we functionalized the sensor with virus protein from the Aleutian Disease Virus (ADV) [19] and then tested the response with serum samples from healthy and from ADV infected minks. The ADV protein and the serum samples were provided by the Antigen Laboratory of the Research Foundation of the Danish Fur breeders Association, Denmark. The functionalized chip was washed once in normal strength (1x) phosphate buffered saline (PBS) at pH 7.4 (Sigma-Aldrich) by applying 5 µL onto the chip inlet. The buffer filled the channel by capillary forces. After 30 sec the channel was drained by applying a piece of soft tissue paper to the outlet thereby removing all the liquid from the channel. The washing was repeated once using 0.1xPBS. The channel was filled again with 0.1xPBS and after 30 sec the resistance was recorded. Recording time was 100 sec with 1 recording/sec. Then serum from a healthy mink (control serum) diluted 1:100 in 1xPBS was applied. After 30 sec the sample was drained, followed by a washing step and a recording step as previously described. Likewise, serum sample from an infected mink was applied (positive serum). A resistance change of approximately 50% when comparing serum from infected animals with serum from

healthy animals (fig. 3b) was detected demonstrating the ability of the NW to detect ADV in minks.

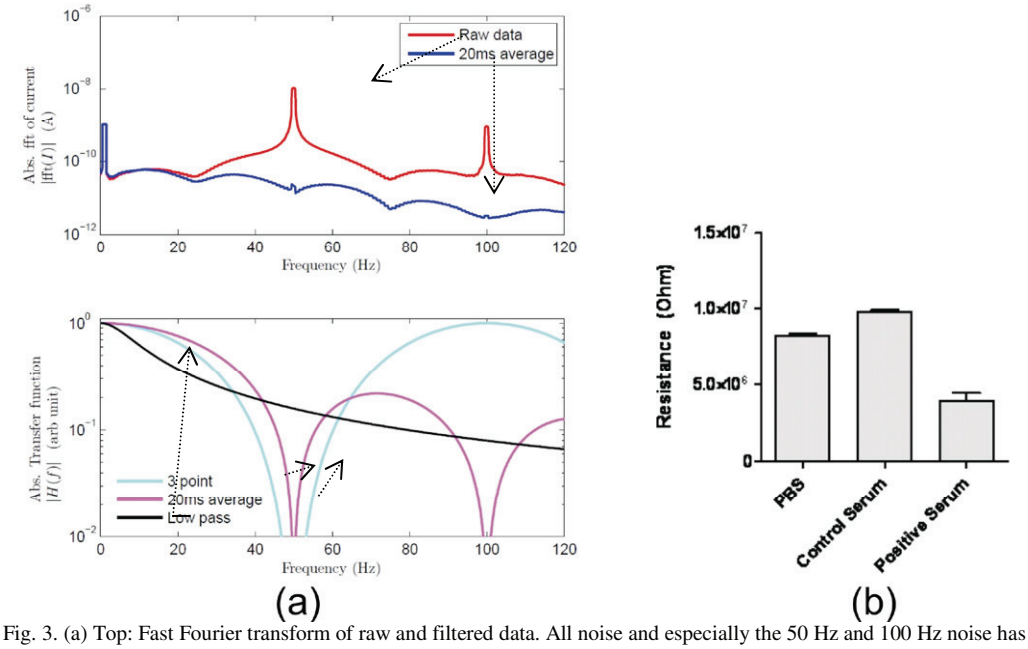


Fig. 3. (a) Top: Fast Fourier transform of raw and filtered data. All noise and especially the 50 Hz and 100 Hz noise has been reduced. Bottom: Modulus of the transfer function of a 3-point filter (removing 50 Hz noise), the 20 ms moving average filter (removing 50 Hz and 100 Hz noise) and the LPF (not removing 50 Hz and 100 Hz noise). (b) The mean resistance (Ohm) (+SD) of the NW from one experiment as recorded over the course of 100 sec with 1 recording/sec for the three solutions.

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