Collaborative project*

Project acronym: SNM

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Deliverable: D8.6 (“Silicon nanowire based FETS as biochemical sensors’’)

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SNM Work Package 8 Deliverable: 8.6 ("Silicon nanowire based FETS as biochemical sensors")										
Lead beneficiary number	7	**Nature**		R		**Dissemination level**		PU		
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Person-months by partner for the Deliverable	CSIC-Madrid									
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Criteria and Achieved Results	**Criteria**	**Achieved result**
	Fabrication of sub-15 nm thick Si nanowire (SiNW) field-effect transistors as biomolecular sensors	Changes of the current or resistance of the device as a function of the biochemical environment
	Lable-free Silicon nanowire FET for detection of a protein species (ferritin)	Changes in the current of the device as a function of the concentration of ferritin
	Silicon nanowire FET for detection of immunological interactions	Changes in the current of the device associated with the interaction between an antigen (BSA) and antibody (anti-BSA)
	Detection limit near single-molecule	About 10 proteins deposited on the SiNW have been detected

Description of the Deliverable

•Fabrication of a SiNW field-effect transistor

This deliverable implements some of the SPL methods described in WP3 to design and generate silicon nanowire field effect transistors as label-free biochemical sensors. The fabrication of silicon nanowire (SiNW) field effect transistors (FET) by oxidation scanning probe lithography (o-SPL) relies on the transfer of an oxide pattern (mask) into the active layer of a silicon on insulator (SOI) substrate by dry etching techniques. Oxidation SPL is used to fabricate extremely thin silicon oxide masks of about 1 nm on top of the active layer of a silicon-on-insulator (SOI) substrate. The etching rate and selectivity is controlled by acting on the proportion of oxygen and the chamber pressure. The complete fabrication process has been described in deliverable D8.3.

Figure 1 illustrates the main lithographic steps to generate a SiNW field-effect transistor from a bare SOI substrate. Once the lithographic steps are completed, the device is annealed by applying several thermal pulses of 300 ^{o}C for 30 s in a N_2 atmosphere. The annealing process improves the electrical response of the SiNW by suppressing the electronic defects generated at the metal–semiconductor contacts during some of the fabrication steps.

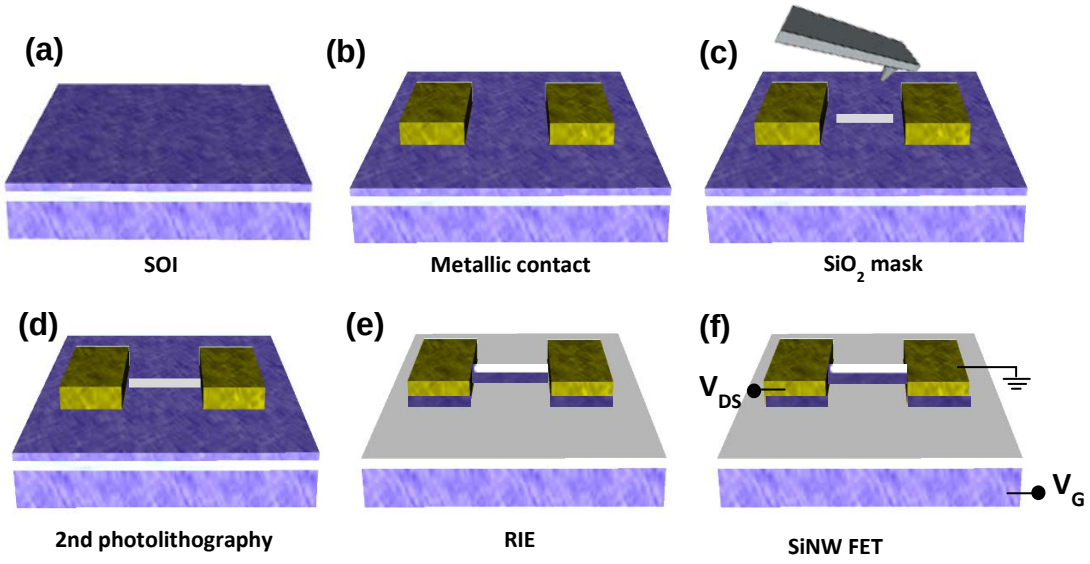

Figure 1. Scheme of the fabrication of a SiNW FET by o-SPL. (a) Silicon-on-insulator substrate. (b) Positioning of the metallic electrodes (photolithography). (c) Local oxide mask generated by o-SPL. (d) A second photolithography step facilitates the contact between the oxide mask and the electrodes. (e) Reactive ion etching is applied to remove the unmasked Si. After this step, the Si region covered by the oxide mask will emerge as the SiNW. (f) The SiNW operates as a back-gated FET.

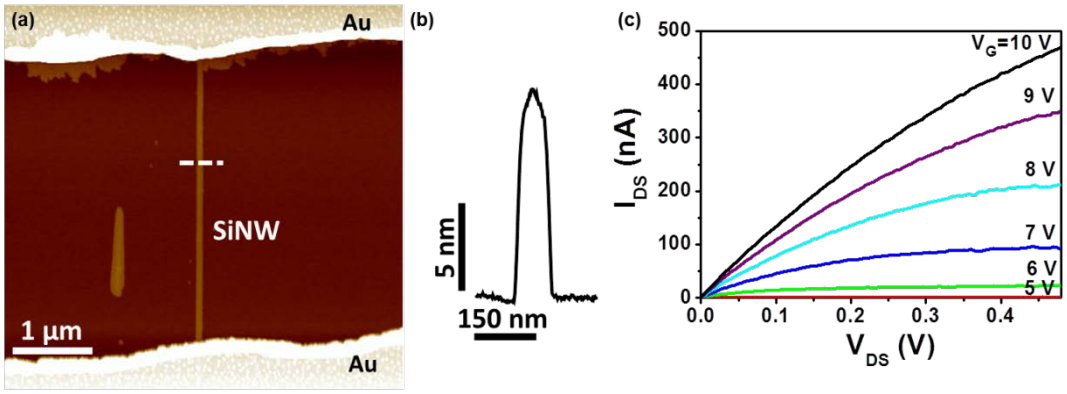

Figure 2. (a) AFM image of a SiNW bridging two Au electrodes. (b) Height profile of the SiNW across the marked line shown in (a). The width and height of the nanowire are, respectively, 75 nm (FWHM) and 12 nm. (c) Output I-V curves of the SINW FET.

Figure 2(a) shows an AFM image of a SiNW FET characterized by length *l*, apparent width *w* and thickness *h,* respectively of *l*= 4.2 *µ*m, *w*= 75 nm and *h* = 12 nm. Figure 2(b) shows its output I-V curves. By increasing the gate voltage (positive values) the current between source and drain increases. This behavior indicates the n-channel character of the SiNW field effect transistors fabricated here.

The subthreshold swing values obtained from the transfer curves of the SiNW FET are in the 200-300 mV/dec range. Those values are dominated by the thickness of the silicon oxide layer of the SOI (25 nm). In any case, those values are close to the theoretical limit obtained for the geometry of the SINWs. This indicates that the lithographic processes do not introduce any significant modification in the properties of the active layer of the SOI substrate.

- **Silicon nanowire biosensor: Encapsulation and Operation principle**

To operate the SiNW devices described above as label-free biochemical sensors requires some additional steps such as the encapsulation of the metallic electrodes to prevent unwanted electrochemical reactions. Those reactions are likely to happen because of the electric bias and the presence of charged species in the solution. Unwanted electrochemical reactions could modify the electrodes and, more importantly, introduce a spurious electrical current between source and drain electrodes.

Here we have covered the source and drain electrodes with a Polydimethylsiloxane (PDMS) layer. This encapsulation protocol isolates the metallic electrodes from the

liquid. It also leaves a microfluidic channel of a few microns in width for the liquid to flow. The SiNW is inside the microfluidic channel. Figure 3 shows a scheme of the device and an optical image of a SiNW biochemical sensor. The gold electrodes, the PDMS layer, the microfluidic channel and the SiNW are marked in the image.

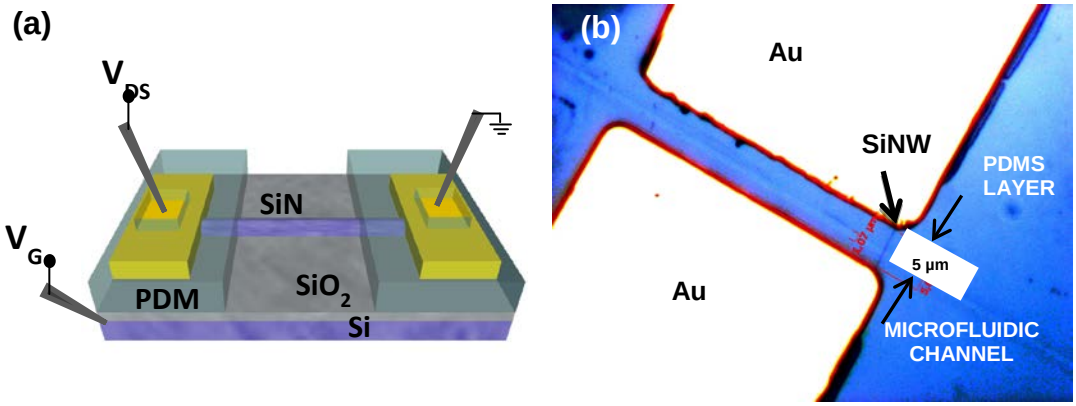

Figure 3. (a) Scheme of the encapsulated SiNW device. A PDMS film covers the metallic electrodes. It leaves a microfluidic channel between the electrodes. (b) Optical microscope image of a SiNW biosensor. The position of the SiNW is marked on the lower right corner.

The first test of the SiNW device to be operated as a label-free biochemical sensor is to detect changes in the pH of the solution. This experiment is relevant for two reasons. First, it serves as an indication of the biochemical sensing capabilities of the nanoelectronic biosensor. Second, it illustrates the sensing principle of the SiNW biosensor. The experiment has been performed by measuring the current through the nanowire as a function of time while the liquid in the microfluidic channel alternates neutral, acid and basic solutions, respectively, at pH values of 5, 4 and 9.

Figure 4(a) shows a scheme of a cross-section of a SiNW biochemical sensor. Figure 4(b) shows that the current between source and drain increases by decreasing the pH of the solution surrounding the SiNW from pH=7 to pH=4. On the other hand, the current decreases by increasing the pH from neutral pH=7 to basic conditions pH=9. Decreasing the pH implies an increase of the concentration of positive species [H^+ and H_3O^+] in the solution. This effect is equivalent to the application of an effective positive voltage gate to the nanowire. Because the SiNW device operates as a n-channel FET, the current increases whenever the medium surrounding the nanowire becomes positively charged. It is important to remark that the biosensing principle of the FET does not imply any overall change in the charge of the solution. The key point is the electrical environment surrounding the nanowire.

The data also shows the stability of the device. Every time that a neutral solution

baths the SiNW, the current flowing the device is the same with independency of the history (Fig. 4(b)).

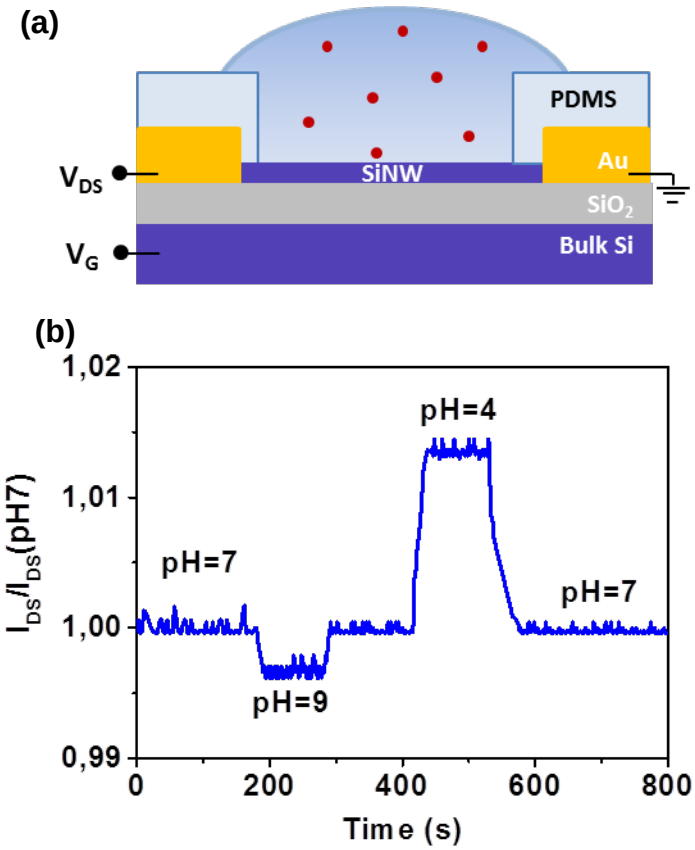

Figure 4. (a) Scheme of the SiNW biochemical sensor. (b) Detection of the pH changes. The current between the source and the drain depends on the pH of the liquid surrounding the SiNW. The current decreases by increasing the pH.. This effect is consistent with the n-channel character of the nanowire FET.

- **Silicon nanowire biosensor: Detection of ferritin**

Ferritin is a cage-shaped protein that accommodates an iron oxyhydroxide nanoparticle. The protein is formed by a polypeptidic hollow shell of about 12 nm in diameter that encapsulates the iron-based core (about 7 nm in diameter). Ferritin has an isolectric point (pI) of 5.4. This means that in a buffered solution with a pH above that value the ferritin will be negatively charged. For a pH value below the isolectric point, the ferritin shows a net positive charge.

To prepare the ferritin suspension we start with an initial concentration of 85 mg/mL in a 0.15 M NaCl solution. Then, the initial concentration is diluted in deionized water by mixing 5 µL of the starting solution in 1 mL of water.

Figure 5 compares the surface of some SiNWs before and after the adsorption of ferritin molecules. For that purpose the surface of the SiNWs has been functionalized with aminopropyltriethoxysilane (APTES). The functionalization protocol has been described in deliverable 3.3. The ferritin molecules appear as rounded structures on top of the SiNWs. Figure 5(b) shows that the proteins are preferentially deposited on the nanowires. The figure also illustrates that the number of molecules acting on the device could be directly counted from the AFM image. On average we find a ferritin molecule every 20 nm along the nanowire.

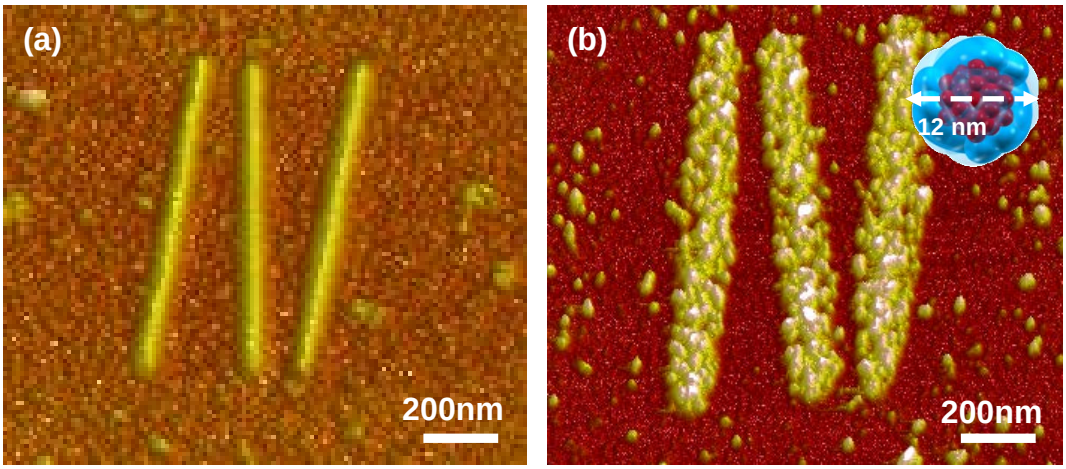

Figure 5. (a) AFM image of three SiNWs. Those nanowires have not been connected to metallic electrodes. (b) The same SiNWs covered with ferritin molecules.

Figure 6(a) shows the operation of the SiNW biosensor in the presence of a suspension containing ferritin molecules. The current was continuously monitored for 5 minutes while different suspensions were introduced in the microfluidic channel. The measurement starts with the current through the SiNW in the dry state (no solution). This is followed by filling the microfluidic channel with the buffer (0.15 M NaCl solution). Finally, the buffer is replaced by a suspension that contains ferritin in a concentration of 425 µg/mL or 1.8 µM (pH=5). The graph illustrates the temporal stability of the device as well as its capability for detecting the presence of the ferritin. In the presence of the protein the current from source to drain increases from 0.5 nA to 2.5 nA. At pH=5 the ferritin is positively charged. The overall effect of the ferritin adsorbed on the nanowire is to act as a positive gate voltage for the n-channel transistor.

Figure 6(b) shows that the response of the SiNW transistor depends on the ferritin concentration. The concentration has been changed from 22 pM to 0.85 µM. The data shows that the higher the concentration, the higher the increase of the current with respect to the value measured in initial solution (buffer without ferritin). This observation correlates the number of ferritin molecules that are either adsorbed on and in the vicinity of the SiNW.

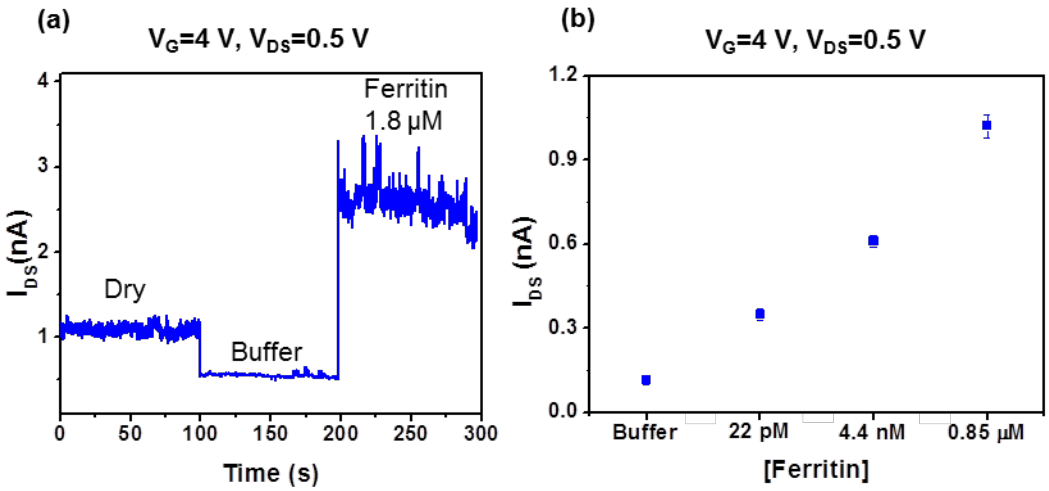

Figure 6. Performance of SiNW biosensor to detect ferritin. (a) Source to drain current for different environments. (b) Source to drain current for different ferritin concentrations. Different SiNWs have been used in panels (a) and (b).

• Silicon nanowire biosensor: Immunological detection

We have also applied the SiNW biochemical sensors to detect immunological interactions. The interaction of a protein, bovine serum albumina (BSA) and its antibody (anti-BSA) has been detected by the SiNW biochemical sensor. The interaction of BSA and its IgG antibody is an example of an immunological interaction. Figure 7(a) shows the scheme of the functionalization of the nanowire with the antibody and the subsequent interaction with the antigen. Figure 7(b) shows the changes in the current between source and drain electrodes as a function of the biomolecular species in the liquid surrounding the nanowire. The current increases by a factor 3 with respect to initial current (dry environment) upon the presence of anti-BSA proteins in the vicinity of the SiNW. The addition of BSA molecules (1 µg/mL) in the microfluidic channel also increases the current by a factor 2 with respect to the level in the presence of the anti-BSA. We have also

performed experiments by reversing the order of the biomolecular species interacting with the nanowire. First by introducing BSA in the microfluidic channel and then by adding anti-BSA. A similar trend was observed (Fig. 7(b)). We note that two different nanowires have been used. That explains the differences in the current measurements.

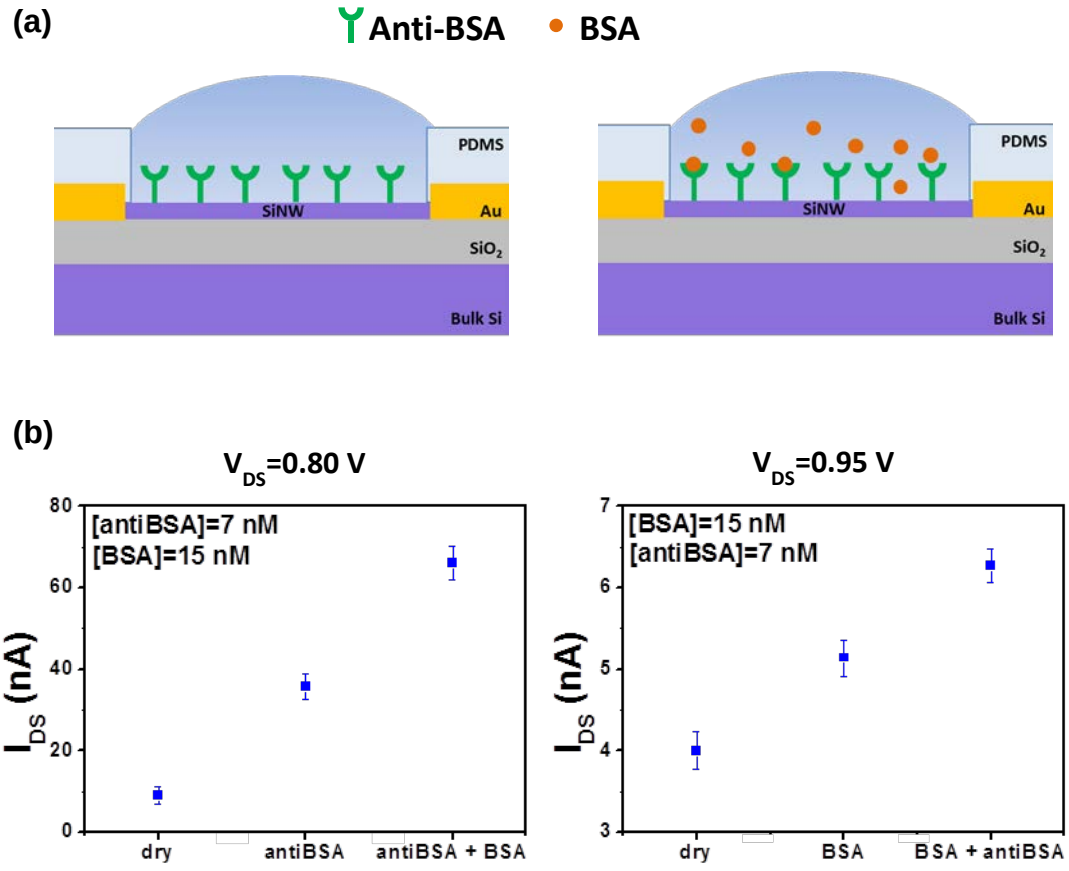

Figure 7. (a) Scheme of a SiNW biosensor to detect immunological interactions (BSA-antiBSA). (b) Current through the SiNW as a function of the different chemical species in the microfludic channel. Different SiNWs have been used in (a) and (b).

The above SiNWs biosensors can be used in several trials. To test the re-usability of the devices we have studied the dependence of the current through the same SiNW after exchanging the liquid in the microfluidic cell several times. First the device is measured in the dry state, then a suspension with anti-BSA followed by BSA was passed through the

microfluidic channel. Then the device was cleaned and measured in the dry state. The process was repeated three times. Figure 8 shows that in the dry state the current through the SiNW remains practically constant at around 3.8 nA. In the presence of biomolecules, the source-to-drain current shows some small fluctuations around 5 nA.

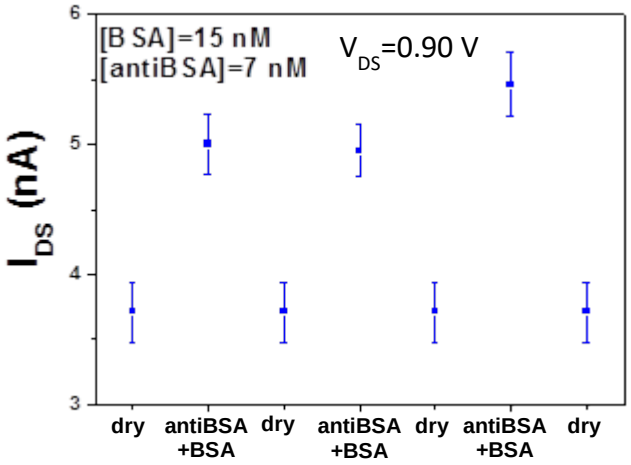

Figure 8. Reversible biosensing operation. Current through the SiNW as a function of the different chemical species in the microfludic channel.

• Silicon nanowire biosensor: Number of molecules detected

Currently there is not a theoretical analysis that can determine the number of molecules detected by the SiNWs as a function of the output or transfer curves. To estimate the number of molecules involved in the detection process we have counted them by acquiring AFM images of the devices. We have also studied the adsorption of the molecules for different concentrations and deposition times on different test samples. This process is suitable for ferritin because its shape and size is easily recognized by AFM imaging. The method is harder to apply for the immunological sensors because the biomolecules are smaller and softer that the ferritin. Based on this method we estimate that at the lowest concentration used here (22 pM) about 10 ferritin molecules are deposited and detected on the SiNW. Figure 9 shows that for low concentration suspensions the number of ferritin molecules adsorbed on the nanowires is very small. This result is in contrast with the data shown in Fig. 5b. That AFM image corresponds to the adsorption of ferritin on the patterns from a concentration of 850 nM.

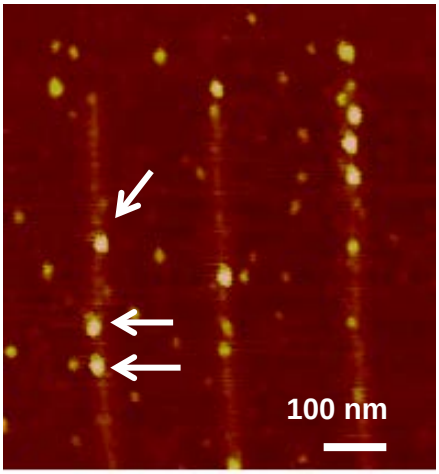

Figure 9. Ferritin adsortion on Si oxide masks at very small concetrations. A 2 µl drop of a suspension of 4.4 nM of ferritin molecules was deposited for 30 s. A discrete number of molecules are observed on the patterns. Some of the ferritin are marked by arrows.

Explanation of Differences between Estimation and Realisation	The operation of silicon nanowires fabricated by o-SPL as label-free biochemical sensors has been demonstrated. The SiNW biosensors devices are very sensitive. We have estimated that at the smallest concentration about 10 ferritin molecules have been detected by the device.
Metrology comments	Issues regarding oxidation SPL metrology have been described in Deliverable 3.4.