

## **Wireless Bacteria Nanosensor Based on Carbon Nanotubes Functionalized with Ag Nanoparticles**

**Nelson I. Alvarez-Colón**, André Marra, Giovanni Caro, Axel Arroyo, Abelardo Colón, Paola Jirau, Sandra Rodríguez, Charlyne Cuyar, Javier Avalos, Gerardo Morell, Brad Weiner

Department of Biology, University of Puerto Rico, San Juan, PR 00936  
Department of Physics, University of Puerto Rico, San Juan, PR 00936  
Department of Physics, University of Puerto Rico, Bayamon, PR 00936  
Department of Chemistry, University of Puerto Rico, San Juan, PR 0093

### **Abstract**

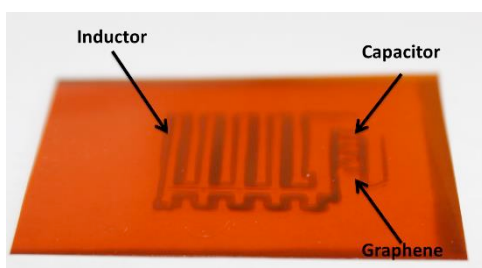
Health communities need a more effective electronic bio-interface to better reduce and eliminate bacterial threat. The biosensor industry fabricates solid devices that are not suitable for biological surfaces such as buccal, skin or any other humid surface. The biological surfaces mentioned above are irregular, for that reason the sensor must possess the ability to attach conformably to such surfaces. In order to solve this problem, we propose a flexible resonance wireless sensor that will detect bacteria and their population density. The bio-sensor consists of a printed radiofrequency wireless sensor made of gold, to which carbon nanotubes and peptides were incorporated. A simple method is devised to deposit highly monodispersed Ag nanoparticles on multi-walled carbon nanotubes (CNTs), which started from an initial modification of Ag nanoparticles. This part of the sensor is attached to a Kapton substrate; this device will neither work with any batteries nor use wire to transfer the information from the bio-sensor to another device. The Kapton based substrate will allow the biotransfer of the sensors onto the biomaterials in a simple and efficient manner. This innovative platform to build nanosensors for biomedical applications will improve bioelectronics for interface monitoring. This project has an importance with the agency of

NASA because it helps to develop different methods for the biomedical sciences and hospitals.

### **Introduction**

Hospital and health communities need a more effective electronic bio-interface to better reduce or eliminate bacterial threat. The biosensor industry fabricates solid devices that are not suitable for biological surfaces such as buccal gums, skin or any other humid surface. The biological surfaces mentioned above are irregular, for that reason the sensor must possess the ability to attach conformably to such surfaces. In order to solve this problem, we propose a flexible resonance wireless sensor that will detect bacteria and their population density. The bio-sensor consists of a printed radiofrequency wireless sensor made of gold, to which carbon nanotubes, and silver nanoparticles will be incorporated. This part of the sensor will be attached into a Kapton substrate; this device will neither work with any batteries nor use wire to transfer the information from the bio-sensor to another device. The Kapton based substrate will allow the biotransfer of the sensors onto the biomaterials in a simple and efficient manner. This innovative platform to build nanosensors for biomedical applications will improve bioelectronics for interface monitoring.

This nanosensor is a LC singer-layer resonance circuit is made with 50nm gold film, the LC-circuit is in parallel with a multi flakes graphene sheet with rough topography. With this basic component of the LC-circuit we form the basic wireless sensor set-up. The Agilent 8712ES RF network Analyzer is connected to one coil antenna that will send the frequency (emitter) and a second coil antenna that will receive the frequency (works as a receiver). The signal analyzer receiver will give power to the remote passive sensor via transmitting all via inductive coupling (Figure 1). Passing an AC signal through the antenna generated a magnetic field, inducing current via mutual inductance in the coil of the sensing element (Faraday's law), and finally resulting in a potential drop that depended on the conductance of the graphene nanosensor.



**Figure 1:** In this drawing we can observe the different parts of the nanosensor.

## Methodology

### 2.1 Nanosensor Design and Creation

In order to select the most efficient design for the nanosensor, three models were created using the program Sketch Up Pro. The three designs consisted of a triangular structure, a small conventional square structure and lastly the design that was selected which is a redesign on the typical rectangular nanosensor. Afterwards, the molds for these designs were built with

the use of a 3D Printer Makerbot. Lastly the molds were used to put the 50 nm gold through the sputtering technique on top of the Kapton substrate.

### 2.2 Carbon Nanotubes and Ag nanoparticles Depositionon Nanosensor

After the nanosensor design had been created in gold the process of adhering depositing the carbon nanotubes on top of the capacitor was initiated. The protocol for this part, first consisted of cutting and flattening the copper foil to a reduced size that matched that of the capacitor in the nanosensor. Afterwards, the copper foil strips were taken to the Nitric Oxide bath in order to remove debris. Then it was taken to an Iron Chloride bath in a petri plate on a hot plate to 55 C. Then we cleaned the nanosensor with a special mix of Acetone, Isopropanol and Alcohol diluted in deionized water and dried the nanosensor with dry air nozzle located in the hood and repeated the process until nanosensor is hydrophilic. Filled 3 beakers with deionized water and took out the carbon nanotubes films from the Iron Chloride bath and placed them in the water. Heat hot plate to 45 C. Placed nanosensor in water and placed carbon nanotubes film on top of the capacitor. Finally, we left the nanosensor with carbon nanotubes film in heated hot plate overnight.

### 2.3. Treatment for Adhesion of Graphene

First we pre-tested that Benzene is not harmful to Nanosensor. Then we heated the hot plate to 161 C. Placed 1 drop of Benzene on the capacitor and dried the Benzene on top of the hot plate without touching the surface of the hot plate. The procedure was repeated 3 times or until the Carbon Nanotubes is no longer visible. Then left overnight on hot plate at 45 C.

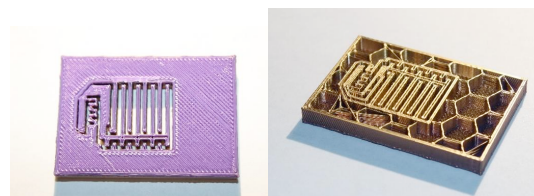
## 2.4 Process for Removing the Polymer

In order to remove the polymer, form the carbon nanotubes we delicately, submerged nanosensor in a beaker with 25mL of acetone multiple times. At first, the area of the polymer will become white which is how we know the process was efficient. Then we heated the hot plate to 202 C and dried the acetone on top of the hot plate without touching the surface of the hot plate. Repeated steps until the polymer was completely removed.

## 2.5 Bacterial Protocol

After the 50nm gold film with functionalized carbon nanotubes. Some images were taken with the IFN (Figure 3) in order to assess how accurate the creation process had been. Once we were sure of the fidelity of the nanosensor to the proposed structure the bacterial protocol was initiated. For this experiment the bacteria selected was Microbiologics™ *Escherichia coli* ATCC® 25922 lyophilized pellets that were inoculated with 5mL following the standard procedure stated in the Microbiologics™ manual. After 24 hours the inoculated *E. coli* was washed with 2.5 ml of PBS Buffer and centrifuged for 10 minutes at 3,900 RPM. This step was repeated three times to ensure that the bacteria had no residual growth medium proteins that could interfere with their attachment to the carbon nanotubes nanosensor. Then a UV Spectrophotometer was used to quantify bacterial concentration at 640 nm. Once the bacterial concentration was known calculation were made to adjust it to 1 OD of *E. coli* which is equal to  $8 \times 10^8$  cell/mL and 2 OD. Lastly 0.25μm of both bacterial concentrations were added to their respective nanosensors and left for an hour to ensure proper attachment of *E. coli*. In the

end we were left with eight nanosensors that consisted of: control nanosensor with no carbon nanotubes, bacteria and then with 1 OD and 2 OD of *E. coli*; control carbon nanotubes with no bacteria and then with 1 OD of bacteria; and carbon nanotubes nanosensor with bacteria and then with 1 OD and 2 OD of *E. coli*.



**Figure 2:** In these pictures we can see (left) the mold created with the 3D Printer and (right) the mold after the process of Sputtering.

## 2.7 Change in Phase Measurements

After the bacteria had been incorporated to the different nanosensors they were taken to the electronics lab in the University of Puerto Rico, Bayamon campus and tested. The set up for testing consisted of an emitter which was an Agilent 8712ES RF network Analyzer connected to one coil antenna that sent the frequency to a receiver which was a second coil antenna. The signal analyzer receiver gave power to our remote passive nanosensor via inductive coupling. Passing an AC signal through the antenna generated a magnetic field, inducing current via mutual inductance in the coil of the sensing element (Faraday's law). This resulted in a measurable change in phase that depended on the conductance of the graphene nanosensor. Each nanosensor was tested three times in order to assure accuracy of the results and then the results were analyzed for statistical significance using a one-way ANOVA and a t-Test: Two Sample Assuming Equal Variances.