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# ANALYSIS STUDY OF SINGLE MOLECULE HPV DNA THROUGH THE GOLD NANOPARTICLE SYSTEM BY USING ATOMIC FORCE MICROSCOPY

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**ABSTRACT**

This paper present study of single molecule Human Papillomavirus (HPV) DNA through the gold nanoparticle system by using atomic force microscopy. Transduction of biorecognition events into electrical signals through the integration of single biomolecule HPV DNA probe in bioelectronic nanodevices requires both a reliable electrical contact and the metallic electrode for efficient conduction mechanism. These conditions have been met in the hybrid system obtained by linking gold nanoparticles with DNA probe. Such an assembling strategy, combined with a conductive atomic force microscopy (AFM) investigation, has allowed us to put into evidence an unprecedented matching between current and topography features and to attribute the intramolecular charge transport. Nanoparticles have been explored as signalling probes for Ultrasensitive DNA detection that can be used in field applications to overcome these limitations. Gold nanoparticles (AuNPs) have been extensively used mainly because of its optical property and ability to get functionalized with a variety of biomolecules. The study demonstrates the use of gold nanoparticles functionalized with single stranded oligonucleotide (GNP- oligo probe) as visual detection probes for rapid and specific detection of Human Papillomavirus (HPV).

**Keywords:** nanodevice, AFM, human papillomavirus, GNP, biomolecule.

## INTRODUCTION

Nanoparticles have been explored widely as signalling probes for Ultrasensitive DNA detection that can be used in field applications. Gold nanoparticles (AuNPs) have been extensively used for biomolecule detection by many research groups mainly because of optical properties and ability to functionalize with a variety of biomolecules (Zanoli, D'Agata, and Spoto, 2012). Gold was the first metal to be transformed into a colloidal state. The colloidal AuNPs are used in the development of several biodetection schemes. Protein-coated gold colloids have been used extensively in lateral flow immunoassay based analytical techniques their application towards DNA detection (Parolo, de la Escosura-Muñiz, and Merkoçi, 2013).

GNPs are the nanostructures most widely used for DNA detection(Jelveh and Chithrani, 2011). Simple synthetic procedures are required in order to obtain GNPs with well-controlled diameters, shapes, and optical properties (Tsai *et al*., 2008). GNPs possess extremely high extinction coefficients, so slight aggregation may result in intense color changes (Chen and Chang, 2004). The large surface area of nanoparticles allows hundreds of capture probe DNAs to be loaded, while the three dimensional assembly of the probe lowers steric hindrance and favors target–probe hybridization (Xu, Pellino, and Knoll, 2008).

Atomic force microscopy (AFM) or scanning force microscopy (SFM) is a very high-resolution type of (SPM) (Sugimoto *et al*., 2007). AFM demonstrated resolution on the order of fractions of a, more than 1000 times better than the (Felts *et al*., 2012). Using an atomic force microscope (AFM), it is possible to measure a roughness of a sample surface at a high resolution (Ando, Uchihashi, and Fukuma, 2008). Besides that, AFM also can be used to distinguish a sample based on its mechanical properties based on hardness and roughness (Webster, Crow, and Fletcher, 2011). In addition, it also was being used to perform a microfabrication of a sample for example, an atomic manipulation (Suzuki, 2000).

In a field of semiconductor physics, for example, (a) an identification of atoms at a surface, (b) evaluation of an interaction between a specific atom and its neighboring atoms and (c) a change in physical properties arisen from a change in an atomic arrangement thorough the atomic manipulation have been studied (Oyabu *et al*., 2005). While in a field of a cellular biology, for example, (a) an attempt to distinguish cancer cells and normal cells based on a hardness of cells and (b) an attempt to evaluate of an interaction between a specific cell and its neighboring cells in a competitive culture system. have been made (Hogan *et al*., 2011).

AFM provides a three-dimensional surface profile (Lee, 2005). In addition, samples viewed by AFM do not require any special treatments such as metal or carbon coatings that would irreversibly change or damage the sample, and does not typically suffer from charging artifacts in the final image. Most AFM modes can work perfectly well in ambient air or even a liquid environment (Baalousha and Lead, 2013). This makes it possible to study biological macromolecules and even living organisms.

The aim of this study was to study the morphology IDE coated with 30nm GNP by using AFM

analysis. IDE thin films with surface modification using GNPs were applied for biomolecule detection of target DNA hybridization.

## MATERIALS AND METHODS

### $SiO_2$ cleaning

$SiO_2$ substrate devices were cleaned for 30 min in a piranha solution consisting of one-third hydrogen peroxide (30%) and two-thirds sulfuric acid (18 M), rinsed in distilled water, left for 10 min in boiling distilled water, dried under an argon flow, and used immediately.

The substrates were then carefully rinsed with de-ionized (DI) water, and dried under a stream of nitrogen gas. The piranha solution (strong oxidizing agent) removes the organic contaminants and makes the surface more hydrophilic by hydroxylating the silicon surface.

### IDE fabrication

The Interdigitated Electrodes (IDE) sensor was fabricated on a silicon-on-insulator (SOI) wafer with a 145nm buried oxide layer (Nadzirah, Ahmad, and Hashim, 2012). Silicon wafer was used as a main substrate in order to form silicon dioxide ($SiO_2$) as an insulation layer of an electrical device. The wafer was cleaned using buffered oxide etchant (BOE) to remove native oxide which had been naturally grow on it. Growth of $SiO_2$ using wet oxidation process provides thicker insulation layer and shorter time consuming compared to dry oxidation process (Adam *et al*., 2012). In order to transfer pattern from a mask on a wafer, photoresist was spin-coated on the growth $SiO_2$ wafer using spin-coater.

Photoresist is a light-sensitive material used to form a patterned coating on a surface. By using deep ultraviolet lithography, a 50 nm silicon layer was patterned and etched. When UV-light was exposed on the phototresist, pattern from chrome mask was directly transfer onto the phototresist. After development process, aluminium metal was deposited using sputter-coater and acetone was used to strip the unwanted photoresist.

### Gold nanoparticle colloid deposition

1µl of GNP (30nm) has been deposited on the active area of IDE for 1 h, at room temperature The active area of Interdigitated Electrodes (IDE) was shown in Figure-1.

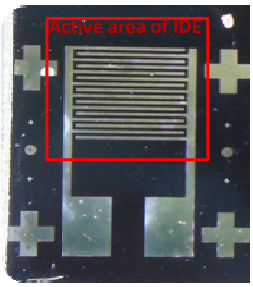


**Figure-1**. Active area of interdigitated device electrode (IDE).

### Surface modification with 3-aminopropyl triethoxysilane (APTES)

The two-dimensional surface is typically prepared by treating the IDE with an 3-Aminopropyl triethoxysilane (APTES) which results in a uniform layer of primary amines (Mamishev, Sundara-Rajan, and Zahn, 2004). The IDE surface was first functionalized using APTES, which functions as a facilitator to immobilize biomolecules on the IDE surface.

### Atomic force spectroscopy

AFM imaging was performed to examine the nature of with slight modifications. Briefly, the test samples after completion of assay were centrifuged at 13000 xg for 30 minutes and the pellet was washed twice in distilled water to remove salt and unhybridized DNA. The pellet was resuspended in 50 µl of distilled water and spread over the poly-l-lysine coated mica sheets and incubated at room temperature for 20 minutes. The excess samples were removed and mica sheets were washed twice with distilled water and allowed to air dry at room temperature. The images were obtained using Scanning probe microscope (NanoscopeIII, Digital Instruments, Santa Barbara, CA, USA) in contact mode on the air using silicon nitrite tips.samples under test condition. The sample for AFM imaging was prepared on these slides as described previously

## RESULTS AND DISCUSSIONS

AFM consists of a sharp tip (probe) at its end that is used to scan the specimen surface was shown in Figure-2. The cantilever is typically or with a tip on the order of nanometers. When the tip is brought into proximity of a sample surface, between the tip and the sample lead to a deflection of the cantilever according to. Typically, the deflection is measured using a spot reflected from the top surface of the cantilever into an array of. Atomic force microscope topographical scan of a glass surface. The micro and nano-scale features of the glass can be observed, portraying the roughness of the material. The image space is (x,y,z) = (20 µm × 20 µm × 420 nm).

The surface topography of the chemically modified silicon substrate was studied using AFM. Tapping mode images were acquired following each step of the immobilization process. The AFM can be operated in a number of modes, depending on the application. In general, possible imaging modes are divided into static (also called contact) modes and a variety of dynamic (non-contact or "tapping") modes where the cantilever is vibrated or oscillated at a given frequency.

To study the surface morphology and roughness of the gnp 30nm on IDE thin films, AFM was conducted to image the IDE thin films over a 1000 nm square area. The top view of IDE coated with 30nm gnp are demonstrated in Figure-3, respectively. To verify the presence of single gold nanoparticle layers on the substrates, 1000x1000 nm areas have been raster scanned at high force load, carefully controlled in order to remove the softer APTES layer without damaging the gold surface.

3-D view of gold nanoparticle (GNP) with 30nm size on IDE thin film was shown in Figure-4. Topography images are stable and reproducible, with no evidence of GNP mobility on IDE surface upon multiple scans, indicating that they are robustly bound. From the tabulated values of AFM data (Figure-5), it could be noticed that GNP/IDE thin films demonstrated the grain sizes (46.37nm), surface roughness (1.609 nm), and RMS roughness (3.325 nm).

Nanoparticles typically fall into one of two categories when it comes to sample preparation. The first category is nanoparticles rigidly attached to a solid structure. The second category is nanoparticles with weak adhesion to the substrate, such as dispersions of nanoparticles in liquid or dry mediums. Sample preparation for the colloidal suspension of nanoparticles was in the second category and involve the stable attachment of particles onto the substrate.

AFM works by scanning a mechanical probe across the sample surface, any structure being imaged must have greater affinity to the flat surface than to probe tip. When nanoparticles do accidentally attach to the probe, the resulting images typically show reduced resolution. Streaking occurred in the images if nanoparticles are not rigidly attached to the flat surface while scanning in contact mode.

AFM has several advantages over SEM or TEM for characterizing nanoparticles. Images from an AFM represent data in three dimensions, so that it is possible to measure the height of the nanoparticles quantitatively. With an AFM, images can be measured in all environments; ambient air, liquids and vacuums. With an AFM, images can be measured in all environments; ambient air, liquids and vacuums. AFM is much simpler to operate than the SEM or TEM so the AFM does not require a specially trained operator.

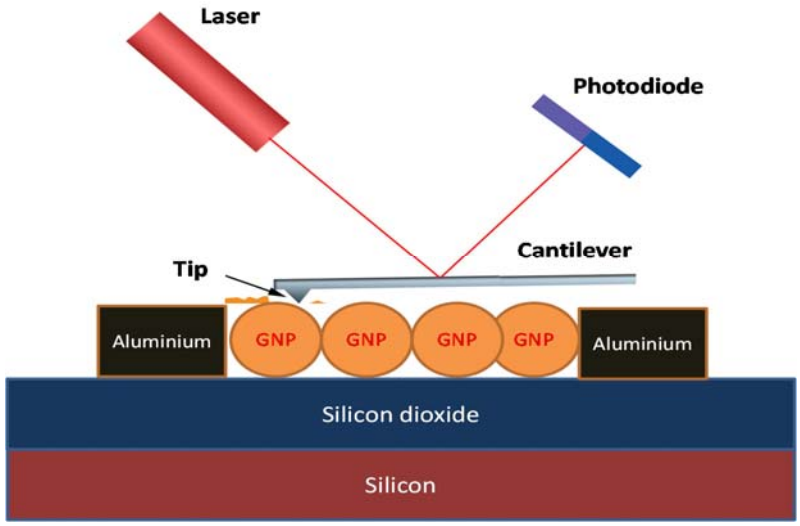


**Figure-2.** Schematic illustration of 30 nm GNPs linked to the top of an APTES monolayer self-assembled on an IDE thin film substrate. Basic principle of Atomic force microscope (AFM). A cantilever, with a very small tip (probe), moves along the surface and experiences atomic forces. Laser and Photodiode are used to measure those forces.

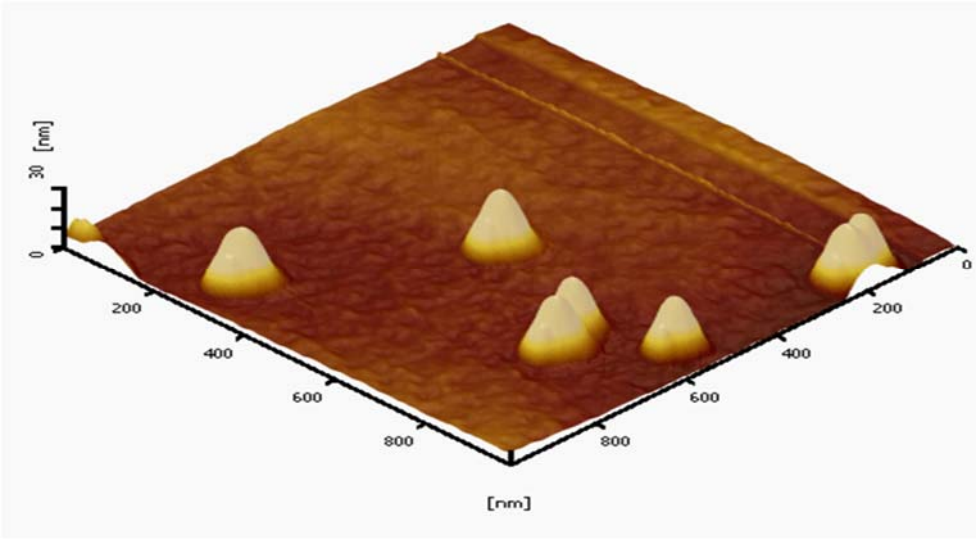


**Figure-3**. 3-D view of gold nanoparticle (GNP) with 30nm size on IDE thin film.

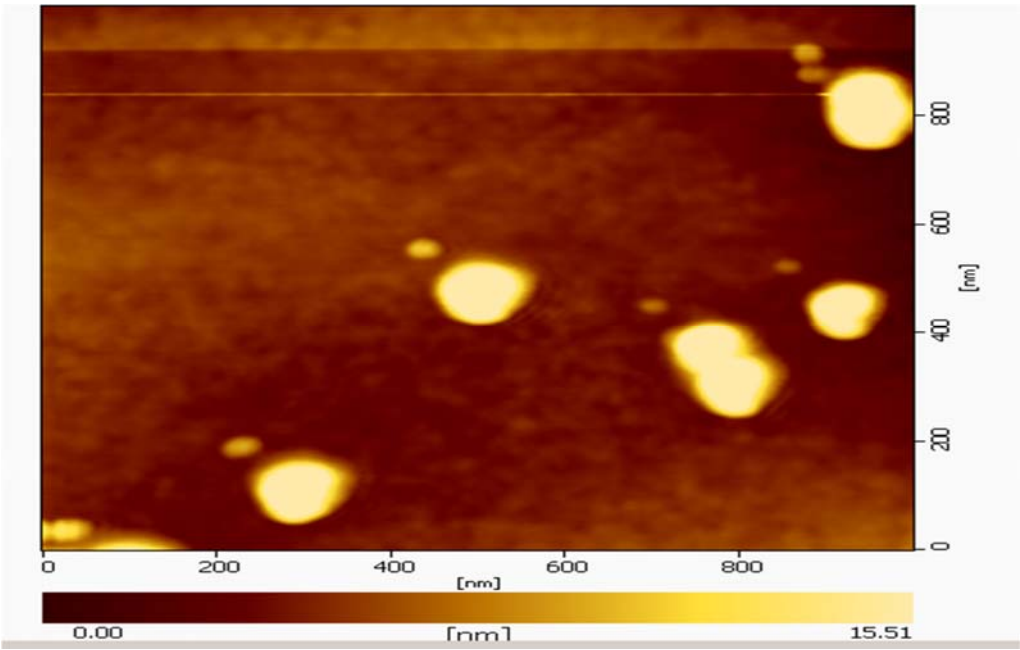


**Figure-4.** Top view of gold nanoparticle (GNP) with 30nm size on IDE thin films.

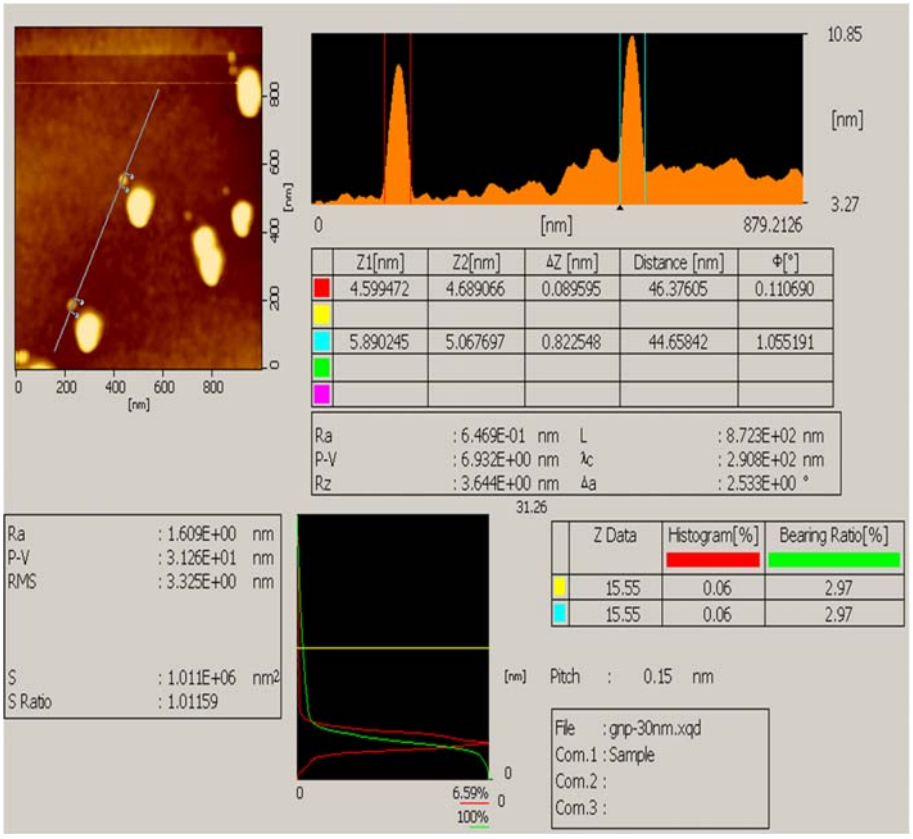


**Figure-5.** Scan area of gold nanoparticle (GNP) with 30nm size on IDE thin films.

## CONCLUSIONS

IDE biosensor was successfully fabricated by depositing APTES and GNPs on the IDE surface. The modified surface with GNPs (35±5 nm) was characterized by Atomic Force Spectroscopy (AFM). The samples were characterized after every step of surface modification and biomolecule immobilization using AFM.

GNP may provide an efficient top electrical contact to IDE thin film substrate, thus enabling to detect a reliable and good electrical current through single molecules and to evidence an unprecedented good matching between AFM topography and current maps. Establishing top contact via GNPs represents a suitable strategy to improve the overall conduction mechanism in hybrid nanojunctions, which, on the other hand, can be easily extended to other type of immobilised biomolecules and could help in building up stable GNP/DNA/electrode hybrid systems to be exploited in biosensing nanodevices.

## REFERENCES

Adam, Tijjani, U. Hashim, Sutikno, Th S. Dhahi, and T. Nazwaa. 2012. "Material Engineering for Nano Structure Formation: Fabrication and Characterization." pp. 361-68 in Procedia Engineering, vol. 50.

Ando, Toshio, Takayuki Uchihashi, and Takeshi Fukuma. 2008. "High-Speed Atomic Force Microscopy for Nano-Visualization of Dynamic Biomolecular Processes." Progress in Surface Science 83:337-437.

Baalousha, M., and J. R. Lead. 2013. "Characterization of Natural and Manufactured Nanoparticles by Atomic Force Microscopy: Effect of Analysis Mode, Environment and Sample Preparation." Colloids and Surfaces A: Physicochemical and Engineering Aspects. 419: 238-47.

Chen, Shih J., and Huan Tsung Chang. 2004. "Nile Red-Adsorbed Gold Nanoparticles for Selective Determination of Thiols Based on Energy Transfer and Aggregation." Analytical Chemistry. 76: 3727-34.

Felts, Jonathan R., Kevin Kjoller, Michael Lo, Craig B. Prater, and William P. King. 2012. "Nanometer-Scale Infrared Spectroscopy of Heterogeneous Polymer Nanostructures Fabricated by Tip-Based Nanofabrication." ACS Nano. 6: 8015-21.

Hogan, Catherine, Mihoko Kajita, Kate Lawrenson, and Yasuyuki Fujita. 2011. "Interactions between Normal and Transformed Epithelial Cells: Their Contributions to Tumourigenesis." International Journal of Biochemistry and Cell Biology. 43:496-503.

Jelveh, Salomeh, and Devika B. Chithrani. 2011. "Gold Nanostructures as a Platform for Combinational Therapy in Future Cancer Therapeutics." Cancers. 3:1081-1110.

Lee, Sunyoung. 2005. "Chemical Functionalization of AFM Cantilevers."

Mamishev, a. V., K. Sundara-Rajan, and M. Zahn. 2004. "Interdigital Sensors and Transducers." Proceedings of the IEEE. 92(5): 808-45. Retrieved (http://ieeexplore.ieee.org/lpdocs/epic03/wrapper.htm arnumber=1288505).

Nadzirah, Sharipah, Syed Ahmad, and U. Hashim. 2012. "Titanium Dioxide Nanowires-Based Interdigitated Electrodes for Biomedical Application." (December): 126-28.

Oyabu, Noriaki, Yoshiaki Sugimoto, Masayuki Abe, Óscar Custance, and Seizo Morita. 2005. "Lateral Manipulation of Single Atoms at Semiconductor Surfaces Using Atomic Force Microscopy." Nanotechnology 16:S112–17.

Parolo, Claudio, Alfredo de la Escosura-Muñiz, and Arben Merkoçi. 2013. "Enhanced Lateral Flow Immunoassay Using Gold Nanoparticles Loaded with Enzymes." Biosensors & bioelectronics. 40:412-16.

Sugimoto, Yoshiaki *et al*. 2007. "Chemical Identification of Individual Surface Atoms by Atomic Force Microscopy." Nature. 446: 64-67.

Suzuki, Hiroaki. 2000. "Microfabrication of Chemical Sensors and Biosensors for Environmental Monitoring." Materials Science and Engineering C 12: 55-61.

Tsai, Shiao Wen *et al*. 2008. "Surface-Modified Gold Nanoparticles with Folic Acid as Optical Probes for Cellular Imaging." Sensors 8:6660-73.

Webster, Kevin D., Ailey Crow, and Daniel A. Fletcher. 2011. "An AFM-Based Stiffness Clamp for Dynamic Control of Rigidity." PLoS ONE 6.

Xu, Fei, August M. Pellino, and Wolfgang Knoll. 2008. "Electrostatic Repulsion and Steric Hindrance Effects of Surface Probe Density on Deoxyribonucleic Acid (DNA)/peptide Nucleic Acid (PNA) Hybridization." Thin Solid Films. 516: 8634-39.

Zanoli, Laura Maria, Roberta D'Agata, and Giuseppe Spoto. 2012. Functionalized Gold Nanoparticles for Ultrasensitive DNA Detection." Analytical and Bioanalytical Chemistry. 402: 1759-71.