SELF-ORDERED GOLD QUANTUM DOTS ARRAY FOR IN VITRO BIOSENSING

Jana DRBOHLAVOVÁ¹, Alexander MOZALEV¹, Vojtěch SVATOŠ¹, Radim HRDÝ¹, Lukáš KALINA², Jaromír HUBÁLEK¹

¹Brno University of Technology, Central European Institute of Technology and FEEC Department of Microelectronics, Brno, Czech Republic, EU, drbohla@feec.vutbr.cz
²Brno University of Technology, Brno, Czech Republic, EU

Abstract

To the best of our knowledge, self-ordered gold quantum dots (Au QDs) array was never fabricated using the approach of gold pulse galvanic deposition into the nanosized dimples, which were created on the special conductive alloy using the nanoporous alumina template. This unique patterning method enables the creation of precisely ordered gold nanostructures with tunable size up to several nanometers. Such nanosized gold array with attractive electrical and optical properties has immense potential in various biomedical application, namely as a diagnostic tool for rapid optical and electrochemical detection of biogenous analytes.

The fabrication process consisted in the anodic oxidation of aluminium layer in the first step, resulting in the alumina template creation, and in the continuous anodization of Ti/W alloy deposited below Al layer on Si wafer in the second step. Subsequently, the anodized tungsten oxide nanostructures were selectively etched off, and gold deposition occurred into the nanodimples via Al₂O₃ template using Au-containing hot electrolyte. In the final step, the alumina template was carefully removed using selective etchant solution.

The stationary polarized fluorescence was used for the study of Au QDs array optical properties, while SEM provided characterization of morphology and topography of fabricated samples. The chemical composition of samples was analysed using EDX and XPS. The preliminary study of Au QDs optical properties change after the interaction of system with tripeptide glutathione (GSH) was also performed.

Keywords: gold nanostructures, biosensing, fluorescence, anodic oxidation, nanoporous template

1. INTRODUCTION

Gold surely belongs to the most attractive materials in the field of sensing and biosensing [1]. Broad scale of biological species can be detected using gold-based biosensors, mostly via electrochemical [2], piezoelectric or optical approach [3]. While majority of biosensor surface modification approaches lie either in the attachment of synthesized colloidal gold nanoparticles [4] or in the creation of gold nanostructures via lithographic techniques, such as electron-beam lithography [5], here we report on direct bottom-up non-lithographic fabrication of biosensing surface modified with strongly fixed gold nanostructures. Our advanced method used to create such nanostructured surface employs the anodic alumina oxide (AAO) nanoporous template and have the advantage of rapidity, reproducibility, low cost and nanostructure size tunability [6, 7]. Precise control of the size and interspacing of gold nanostructures leads to metal nanoarrays with distinct electrochemical and optical properties. The method enables the fabrication of various shaped gold nanostructures, such as nanodots, nanotubes, nanorods, and nanoshells.

Depending on the demanded application, these gold nanomaterials can be functionalized with a range of surface modifiers which for example permit further imaging enhancements [8]. Beside the optical detection, the Au nanostructured surface can find a potential as a platform for electrochemical detection of prokaryotic (bacterial) [9] and eukaryotic cells [10], e.g. identification/quantification and monitoring of proliferation and adherence of tumor cells on the electrode surface [11].
The aim of this research is to fabricate gold nanostructured conductive surface and to study the fluorescence properties of this system functionalized with GSH for potential application in optical biosensing. Such surface can eventually serve can be also used for electrode modification and further in electrochemical biosensing.

2. EXPERIMENTAL

2.1 Gold quantum dots array preparation

The metal layers for nanoporous anodized alumina (AAO) template creation and subsequently WO₃ nanostructures formation were prepared as follows: Al layer with thickness of 185 nm was thermal-evaporated on sputter-deposited W/Ti alloy layer (42 nm) on Si wafer. Anodic oxidation of Al layer into alumina nanoporous template took place in the potentiodynamic regime in aqueous solution of sulphuric acid (Penta, CZ). As soon as the oxide barrier layer of AAO was opened when touching the surface of W layer, WO₃ nanodots started to grow (see Fig. 1 C). In the following step, WO₃ nanostructures were selectively etched off, which resulted in the creation of nanodimpled surface used subsequently for gold pulse galvanic deposition. The deposition of Au QDs was realized in the aqueous electrolyte composed of potassium dicyanoaurate (Safina, CZ) and boric acid (Penta, CZ). Various deposition conditions including the number of pulses, deposition time and relaxation time between each pulse were tested. The final selective etching of AAO template was performed in hot solution of chromium(IV) trioxide and phosphoric acid, both purchased from Penta, CZ.

Fig. 1 Schematic illustration of Au QDs array fabrication: A – Al(blue)/W(black) bilayer; B – formation of nanoporous AAO template; C – W oxidation inside of pores into WO₃ nanodots; D – nanodimpled surface after WO₃ selective etching; E – Au QDs in nanoporous template; F – Au QDs after AAO template removing.

2.2 Gold quantum dots array characterization

The quality of original metal layers determines the final QDs ordering and size distribution. Therefore, the surface roughness of pure metal layers, especially Al, was investigated using profilometry (Taylor Hobson) before the anodization process. The chemical composition of these layers was characterized by XPS (AXIS Ultra DLD, Kratos) combined with depth-profiling ion beam for sputtering of surface impurities.

The size and homogeneity distribution of fabricated AAO template and nanostructures (WO₃ nanodots, W nanodimples, and Au QDs) were estimated using SEM (Mira II MLU, Tescan). EDX was employed for chemical composition analysis of fabricated Au QDs array.

The stationary polarized fluorescence spectroscopy (Fluorolog 3, Horiba Jobin-Yvon) was used for the study of Au QDs array optical properties before and after biofunctionalization. The functionalization was performed with aqueous solution of reduced GSH (Merck, GE), which was immobilized on Au QDs surface by means of physical adsorption via thiol group. The chosen concentration of GSH solution for preliminary study was 10 µg L⁻¹ and the volume deposited onto sample surface corresponded to 18 µL.
3. RESULTS AND DISCUSSION

3.1 Profilometry characterization

The results from profilometry measurement (see Fig. 1) indicated that Al layer is suitable surface for preparation of AAO template, because the surface roughness average was found to be only 0.4 nm. The size of Al grains was about 100—150 nm and no homogeneities or cracks appeared in the layer. The layer of tungsten was also flat enough and possessed very low surface roughness with regular grain size.

![Fig. 2 Topography of Al layer from profilometric measurement.](image)

3.2 QDs size and distribution characterization

According to SEM analysis, the size of created nanodimples in W after WO₃ nanodots removing varied from 30 nm to 40 nm when lower concentrated electrolyte was used (see Fig. 3 right) and from 20 nm to 25 nm using electrolyte with higher concentration.

![Fig. 3 SEM image of WO₃ nanodots (left) and tungsten nanodimpled surface after WO₃ nanodots removing with remaining AAO template in the upper image part.](image)
The estimated size of Au QDs according to SEM varied from 10 to 30 nm (see Fig. 4). The higher number of deposition pulses strongly increased the density of W nanodimples coverage with Au QDs. In addition, the duration of deposition slightly influenced the homogeneity of Au coverage as well. Namely, the higher deposition time improved the homogeneity. On contrary, the relaxation time between each pulse did not play a significant role in Au QDs distribution.

Pulse galvanic Au deposition procedure was found to be very delicate process, because the presence of air bubbles in the electrolyte negatively affected its reproducibility. Therefore, the attention has to be paid to the construction of deposition device, mainly to the electrode shape and its placement above the sample, so the bubbles evolution can be eliminated. This was successfully accomplished by usage of Teflon cylindrical cover containing gold cylindrical electrode with conic bottom.

![Fig. 4](image)

**Fig. 4** SEM image of Au QDs deposited at 15 pulses with deposition time of 100 ms (right) and 200 ms (left) after AAO template removing.

### 3.3 Characterization of chemical composition

EDX analysis of samples with Au QDs confirmed the presence of Au, W, Si, Sb (as Si doping material), Ti, Al (trace amount originating from AAO template) and impurities such as C and O. Therefore, further qualitative analysis of metal layer composition was performed using XPS in order to find, if the carbon is presented as surface impurity or in the form of carbide inside the layers. This analysis confirmed only C in the form of surface impurity, because after sputtering of 10 nm from Al layer, the content of C decreased to 0 % from original 24 % and moreover, the content of oxygen decreased to 13 % from 40 %.

All metal layers deposited on the sample, as well as Si wafer alone, presented strong reflective signal, which influenced the fluorescence spectra of pure Au QDs samples. This fact is due to much smaller material quantity of Au QDs compared to bulk metal layers. Regarding this disturbing phenomena, the most suitable wavelength for excitation of samples with Au QDs was 300 nm, which was estimated from excitation 3D scan.

### 3.4 Characterization of QDs fluorescence properties

The fluorescence analysis of pure Au QDs sample prepared at 7 pulses (current of 1 mA, deposition time of 100 ms and relaxation time of 5s) showed that the maximal emission intensity was about $1.4 \times 10^5$ cps with the peak maximum at 370 nm (see Fig. 5). However, after Au QDs surface modification with GSH solution, the intensity was about 15 times higher ($2 \times 10^5$ cps) and the maximum position was slightly shifted to 358 nm.
This phenomena can be explained by passivation of defects on QDs surface or the energy transfer from GSH moieties to QDs, which is analogous to classical Förster energy transfer [12].

The full width at half maximum (FWHM) parameters of unmodified samples regarding various preparation conditions are listed in Table 1.

**Table 1** FWHM parameters of samples with unmodified Au QDs

<table>
<thead>
<tr>
<th>Number of pulses</th>
<th>Duration of pulse (ms)</th>
<th>Pore size in AAO template (nm)</th>
<th>FWHM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>250</td>
<td>20—25</td>
<td>66</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>20—25</td>
<td>65</td>
</tr>
<tr>
<td>15</td>
<td>200</td>
<td>20—25</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>10</td>
<td>63</td>
</tr>
</tbody>
</table>

This parameter did not change significantly with variation of gold deposition conditions. The average value corresponded to 65 nm. The similar FWHM value was observed for GSH modified Au QDs, namely 58 nm.

![Fig. 5](image)

**Fig. 5** Fluorescence spectra of Au QDs deposited at 7 pulses (red) and the same Au QDs after modification with GSH solution (blue).

4. **CONCLUSION**

Array of self-ordered Au QDs with tunable size (in the range of 10–30 nm) were successfully achieved via template based method. These QDs were composed of expected chemical elements with commonly occurring surface impurities such as carbon and oxygen. The fluorescence properties of Au QDs were found in the visible range of spectra with maximum position at about 370 nm and FWHM parameter of about 65 nm. The intensity of fluorescence significantly increased after adsorption of GSH molecules on Au QDs surface. This functionalization procedure enables further Au QDs array usage in biosensing, because GSH can serve as binding partner for detected analyte in the solution.

**ACKNOWLEDGEMENT**

*The research was supported by the project GAČR P102/11/1068 NaNoBioTECell.*
REFERENCES


