Supporting Information

Large Scale Fabrication of Ordered Gold Nanoparticle–Epoxy Surface Nanocomposites and Their Application as Label-free Plasmonic DNA Biosensors

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S1. Solid state dewetting of Au films with various thicknesses on the nanobowled templates

In Figure S1 morphologies of NP arrangements are presented, which were prepared by solid-state dewetting of deposited Au thin films with various thicknesses on the nanobowled templates (type B, 40 V). Deposition of only 2 nm gold results in a uniform layer of small, separated NPs with sizes under 10 nm. Here the arrangement of the NPs does not correspond at all with the geometry defined by the template. Increasing the thickness of the deposited thin film leads to the merging of these small NPs into larger, connected films (patches) visible already at 4 nm thickness. The interparticle spaces are reduced to crack-like voids, which continuously diminish with increasing amount of deposited material. The occurrence of these voids depends on several parameters of the deposition technique (for example the method itself, deposition rate, deposition angle), but also on the substrate morphology or wettability by gold as described in the following section (S2). At 8 nm initial layer thickness the voids are disappeared completely and the particle
arrangement corresponds nicely with the template (the particles sit in the dimples defined by the bowls). Above 8 nm the particles start to merge, forming connections and satellite structures.

Figure S1. SEM images of various thickness of deposited Au films over aluminum template type B (40 V) and corresponding results of the dewetting process (300 °C, 5 min).

These results indicate a very narrow window of film thickness for an ideal dewetting process (a single NP per bowl). As shown in Figure S1 or in more detail in Figure S3.2, for a type B template this narrow window is only 8 ± 0.5 nm. For a type A template, where the bowls are smaller, the best results were achieved with 6 nm of deposited thickness. However, the density of defects is much higher due to the challenging preparation of a compact initial film.

S2. Influence of the native aluminum oxide on the morphology of the deposited thin film

It was experimentally observed that the age of the aluminum templates influence the quality of the prepared nanoparticle arrangements. Here, we illustrate that this effect can be explained by a presence of a native oxide layer. In Figure S2 two examples are shown: a template without native oxide that was etched in the alumina etchant right before (less than 1 h) Au deposition, while a template with a native oxide layer that was left to oxidize after PAA removal in ambient conditions for more than 2 weeks prior to Au deposition.
Figure S2. Comparison of SEM images of the 8 nm Au film deposited on aluminum substrates without (top) and with (bottom) native oxide with corresponding results of the dewetting process (300 °C, 5 min).

The deposition of 8 nm thin film results in a more compact layer for freshly the etched template compared to the one with a thicker native oxide. This is caused by the wetting ability of gold (surface energies) which is much higher for metals than for the corresponding oxides. Voids and defects in both cases occur primarily on the sides of the protrusions. This may be due to material deficiency caused by the shadowing effect during the deposition and/or again poor wettability on the more oxidized sides of the aluminum template.

S3. Influence of the annealing temperature on the resulting NP layers

As shown in Figure S3.1 the annealing temperature does have a significant effect on the formation of the NP arrangement. However, over 300 °C no visible changes can observed. This applies also for different deposited layer thicknesses (shown in Figure S3.2). A minor difference in the form of more confined NPs, prepared at higher temperature and longer annealing time can be noticed for the 10 nm thick initial layer.
Considering also that the formation of thermal oxide over aluminum is temperature dependent, we decided to perform annealing at 300 °C for short time periods on a hotplate. The use of hotplate annealing was due to convenient reasons. However, it has to be noted that vacuum annealing is more suitable to avoid thermal oxidation or other contaminations.

Figure S3.2. SEM images of various thickness of deposited Au film over aluminum template type B (40 V) and corresponding results for two annealing temperatures: on a hotplate at 300 °C for 5 min and at 400 °C for 2 h.
S4. Repeated Au thin film deposition and annealing on the nanobowled templates

Examples of repeated thin film deposition and subsequent annealing, with respect to thickness tuning is shown in Figure S4. The main line (B1, B2, and B3, used for the investigations in the paper) shows the best path of increasing thicknesses, compared to the alternatives which contain more defects (smaller particles in the gaps or merged particles).

![Figure S4](image.png)

**Figure S4.** SEM images of the resulting NP arrangements, prepared by repeated deposition and annealing of Au films with different thicknesses on type B templates.

S5. Thickness estimation of gold thin films

The Au films were deposited by RF magnetron sputtering (BESTEC, magnetron sputtering system). The deposition rate was monitored before depositions by a quartz crystal microbalance placed 174 mm from the target. To estimate deposition rate at the sample position (200 mm) a calibration sample was prepared and the thickness of deposited film was measured by profilometer.
(Dektak XT, Bruker) in center (35.8 ± 1.7 nm) and 50 mm from center (36.1 ± 1.4 nm) to confirm a good uniformity.

Similar layers was achieved by Electron Beam Physical Vapor Deposition (EBPVD) made in a BESTEC electron beam evaporator (shown in Figure S5). Deposition was carried out with a deposition rate of 0.1 nm s\(^{-1}\) monitored by quartz crystal microbalance. In Figure S5 the only main difference between the layers deposited by the two methods is their roughness. This may not play a significant role for type B templates, but can be considerably for the type A templates with smaller bowl sizes.

![Comparison of SEM images of the 8 nm Au film deposited by evaporation and sputtering, and corresponding results of the dewetting process (300 °C, 5 min).](image)

**Figure S5.** Comparison of SEM images of the 8 nm Au film deposited by evaporation and sputtering, and corresponding results of the dewetting process (300 °C, 5 min).

**S6. Estimation of the nanoparticles’ size and distribution**

The NP distribution presented in this work were estimated by using Gwyddion software (version 2.53). For analysis used SEM images of 5 µm view field were used and the main NPs were selected by thresholding, avoiding defects like merged particles or small secondary NPs on the side of cells (an example shown in Figure S6). This gives for statistics over 3000 samples for type A, and over
1300 samples for type B template. The diameter of NPs were taken as the diameter of an equivalent circular grain with the same geometrical area. The template dimensions were analyzed based on the detailed SEM images (1 µm view field) by hand. The statistically relevant average of over 50 measurements were taken for each template type.

**Figure S6.** Example of grain selection on SEM image in Gwyddion software.

**S7. Adhesion tests**

The adhesion of nanoparticle layers transferred to the epoxy substrate was tested by Scotch tape test. For this purpose a tape (8705B, TQC Sheen) for adhesion test was used as illustrated in Figure S7. The test were performed on epoxy samples etched for various times (0–40 s, same from Figure 3) with a negative result: no NPs were transferred to the adhesive tape in any case.
Figure S7. Example of an adhesion test on B1 20 s etched sample: a) sample with the attached adhesive tape, b) tape after peel off next to the sample. In the pictures a colored spot is visible due to a previous XPS analysis (epoxy degradation).

S8. Structural instability after DNA immobilization in high (1 M) ionic strength buffer

As discussed in Chapter 3.3, performing the probe-DNA immobilization in a buffer solution containing 1 M NaCl resulted in the structural instability of the nanoparticle arrangement. In high ionic buffers the number of immobilized DNA on the surface is increased, which means increased charge density that needs to be screened with buffer ions. If the ionic strength of the buffer decreases, the electrostatic repulsion between the DNA strands increases, which can destabilize the NP arrangement, namely remove the NPs from the epoxy support pillars. This can be easily confirmed experimentally: after the immobilization of DNA in 1 M NaCl buffer, washing the surface with lower ionic strength buffer, or DI water causes the disintegration of the nanoparticle arrangement. The NPs can be visibly washed away from the surface. It was found that sometimes even the same buffer (1 M) can remove a small amount of particles during the washing step between immobilization/hybridization or after hybridization. Figure S8 presents optical and SEM images of a sample, where the particle integrity was damaged in the described way. The particles are removed in patches, in the empty spots even the remainders of the narrow epoxy pillars can be seen (b3).
**Figure S8.** Optical microscopy image (a1) and SEM images in various magnification (b1–b3) made on a sample, where the nanoparticle layer was damaged during a washing step after the immobilization of DNA in a high ionic strength buffer (1 M NaCl).

**S9. Assessment of probe-DNA coverage**

To assess the probe-DNA coverage on the sensor surface after immobilization two models were used. The first model presumes an exponential field decay,\textsuperscript{1,2} as in Eq. (1):

\[
\Delta \lambda = S(n_{\text{add}} - n_a) \left( 1 - e^{-\frac{-2d_{\text{add}}}{l_d}} \right).
\]  

(1)
Here, $\Delta \lambda$ is the wavelength shift caused by the deposition of an added layer with $n_{\text{add}}$ refractive index and $d_{\text{add}}$ effective thickness. $n_a$ is the refractive index of the ambient (buffer), $l_d$ is the field decay length, $S$ is the bulk RI sensitivity.

Since the field decay of nanoparticles can be better approximated with a power-law like decay,\textsuperscript{3} we will also use a formula which was offered for spherical nanoparticles (with a radius of $R_0$), as in equations:

\begin{equation}
  n_{\text{eff}} = n_{\text{add}} - \frac{n_{\text{add}} - n_a}{\left(1 + \frac{d_{\text{add}}}{R_0}\right)^2},
\end{equation}

\begin{equation}
  \Delta \lambda = S(n_{\text{eff}} - n_a).
\end{equation}

When $d_{\text{add}}$ is calculated from either Eq. 1 or Eqs. 2–3, the probe density can be estimated as:

\begin{equation}
  P_s = \frac{d_{\text{add}}^2 \frac{dn}{dc}}{d_{\text{add}}^2 \text{MW}}.
\end{equation}

We would like to emphasize, that the calculations based on the two models can be considered as only \textit{rough estimations} for the surface coverage. Both models assume a lot of parameters that are not precisely known, and thus these estimations are very unreliable for the following reasons:

- The exact field decay is not known for our system.
- Our particles are not spherical, but have a highly irregular shape. Here we used the thickness of the particle (length along the normal axis) to estimate $R_0$. For the B3 type nanocomposite – used for the sensor characterizations – $R_0$ is estimated as 26 nm based on SEM images.
- The models do not consider near field coupling between the particles and in our case the effect of coupling can be substantial.
• The models do not take into account the effect of the substrate under the particles.

• The models assume the optical properties of the deposited layers, for example the refractive index of a dense DNA layer, which also introduces uncertainties as pointed out in ref. 4.

Besides calculating the probe coverage, as a control, the parameters of a pure MCH monolayer was tested as well.

The used parameters for the calculations are the following (with references):

Bulk refractive index sensitivity: \( S = 92.6 \text{ nm RIU}^{-1} \) (corresponding to Fig. 6).

Refractive index of ambient buffer (0.75 M NaCl 50 mM Na₂HPO₄): \( n_a = 1.34 \).

Thickness of a pure MCH monolayer: \( d_{\text{add}} = 1.2 \text{ nm} \).

Refractive index of pure MCH: \( n_{\text{add}} = 1.45 \).

Refractive index of pure DNA in buffer: \( n_{\text{add}} = 1.45 \).

The \( dn/dc \) of DNA molecules: \( dn/dc = 0.14 \text{ cm}^3 \text{ g}^{-1} \).

The buffer ion free RI contribution of DNA molecules on the surface per unit layer thickness: \( dn/dd = 5 \cdot 10^{-4} \text{ RIU nm}^{-1} \).

Measured absorbance shift due to a pure MCH monolayer: \( \Delta \lambda = 1.88 \text{ nm} \).

Measured absorbance shift due to probe-DNA immobilization: \( \Delta \lambda = 8.08 \text{ nm} \).

Field decay length (for nanodiscs with the same aspect ratio as our NPs): \( l_d = 30 \text{ nm} \).

Molecular weight of DNA probes: \( \text{MW} = 6307 \text{ Da} \).
Based on these parameters the first model with exponential field decay (Eq. 1) predicts a $\Delta \lambda = 0.8$ nm shift for a pure MCH monolayer, while the second model with power-law decay predicts a $\Delta \lambda = 1.3$ nm shift which is much closer to the measured 1.88 nm. The power-law model, which is more focused on the vicinity of the surface predicts the wavelength shift better. It has to be emphasized again, that both models are very sensitive to the field decay length ($l_d$) or the particle radius ($R_0$), which are only roughly estimated, not to mention the effect of irregular shape and particle coupling.

The resulting probe-DNA surface coverages from the two models are $4.9 \cdot 10^{12}$ molecules cm$^{-2}$ and $2.5 \cdot 10^{12}$ molecules cm$^{-2}$, respectively. The results – although rough estimations – can be considered realistic compared to the literature. For example, the group of Herne, Tarlov and Levicky measured probe coverages with 1 M ionic strength buffer between $3-9 \cdot 10^{12}$ molecules cm$^{-2}$, depending on their measurement method.$^4-^6$

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