High-Resolution Scanning Electron Microscopy (HRSEM) and Atomic Force Microscopy (AFM) in Biosensing

Tatyana Bendikov, Tanya Karakouz, Alexander Vaskevich, and Israel Rubinstein

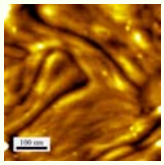
Department of Materials & Interfaces, Weizmann Institute of Science, Rehovot 76100, Israel

In recent years we have developed a new kind of optical transducers based on discontinuous, island-type gold films prepared by evaporation on transparent substrates (e.g., glass). The transduction is based on the sensitivity of the localized surface plasmon resonance (LSPR) absorption band to changes in the effective refractive index in the immediate vicinity of the metal islands. In the present work we show that biorecognition events monitored using the LSPR transducers can be complemented and substantiated by direct imaging of the binding using two microscopies, i.e., HRSEM and AFM. HRSEM and non-contact (AC mode) AFM were applied to visualization of specific binding in two biological systems: protein-protein interactions and DNA hybridization. In addition, contact mode AFM was used for obtaining biological layer thicknesses.

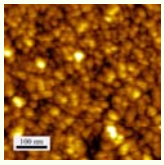
Protein-Protein Interactions

AFM

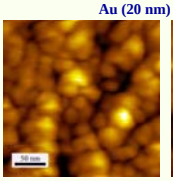
Au (20 nm, annealed)

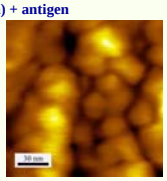


Au (20 nm) + antigen

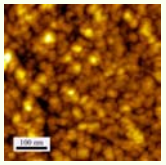


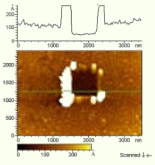
Au (20 nm) + antigen





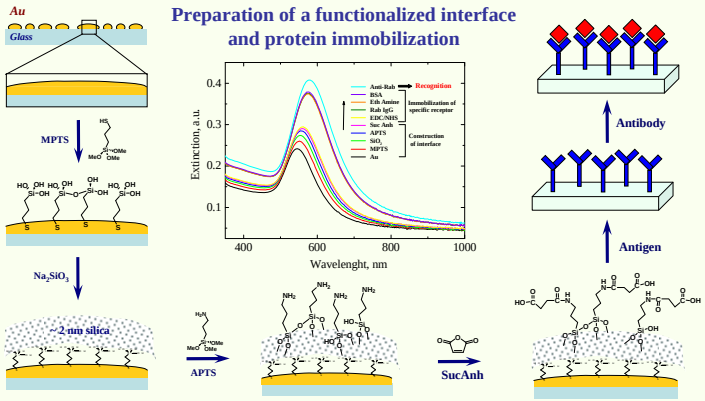
Au (20 nm) + antigen + antibody





Average thickness values of 3.9 ± 0.9 nm for the antigen layer and 8.1 ± 1.2 nm for the antigen + antibody layer were obtained using mechanical abrasion of the sample (multiple scanning in contact mode, window 800×800 nm²).

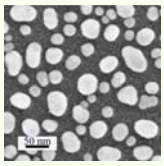
Preparation of a functionalized interface and protein immobilization



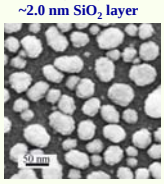
J. Am. Chem. Soc. 129 (2007) 84-92.

HRSEM

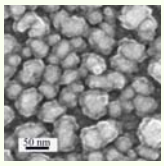
Au (5 nm, annealed)



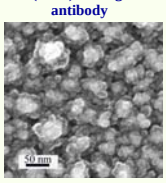
Au (5 nm) + ~2.0 nm SiO_2 layer



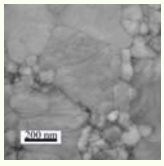
Au (5 nm) + antigen



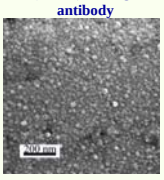
Au (5 nm) + antigen + antibody



Au (20 nm, annealed)



Au (20 nm) + antigen + antibody

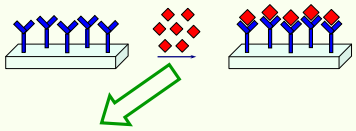


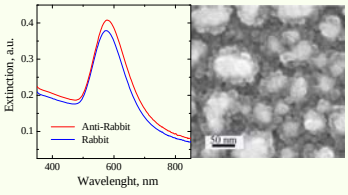
All samples coated with 3 nm Cr.

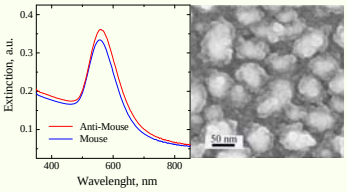
Antigen: Rabbit Immunoglobulin G (IgG), Mouse Immunoglobulin G (IgG)

Antibody: Anti-Rabbit Immunoglobulin G (IgG), Anti-Mouse Immunoglobulin G (IgG)

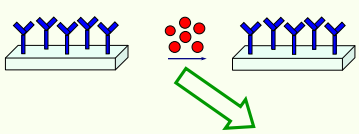
Antigen – Specific antibody

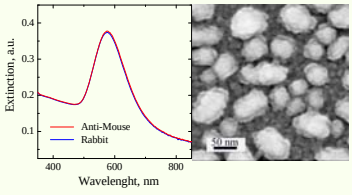


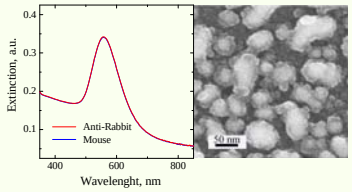




Antigen – Nonspecific antibody







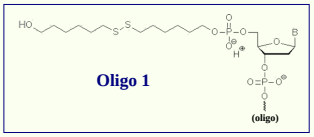
DNA Hybridization

Strands used:

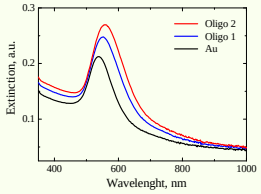
Oligo 1: 43 bases, disulfide modified at 5' end.

Oligo 2: 43 bases, complementary to Oligo 1.

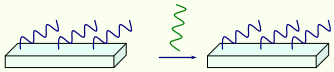
Oligo 3: 43 bases, non complementary to Oligo 1.

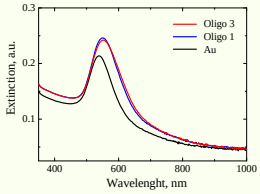


Oligo 1 – Oligo 2



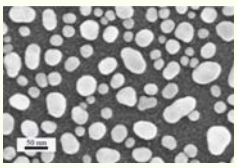
Oligo 1 – Oligo 3



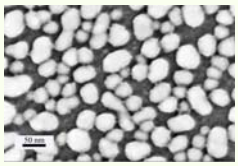


HRSEM

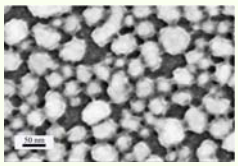
Au (5 nm, annealed)



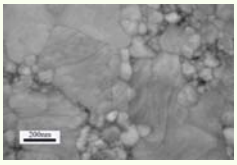
Au (5 nm) + Oligo 1



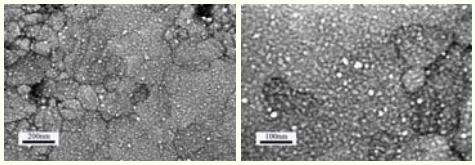
Au (5 nm) + Oligo 1 + Oligo 2



Au (20 nm, annealed)



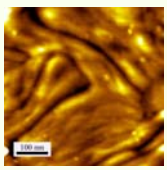
Au (20 nm) + Oligo 1 + Oligo 2



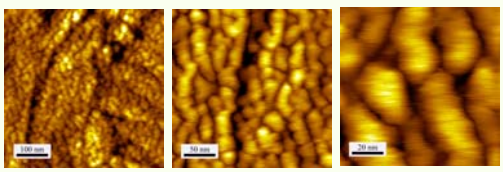
All samples coated with 3 nm Cr.

AFM

Au (20 nm, annealed)



Au (20 nm) + Oligo 1 + Oligo 2



Conclusions

Direct imaging using HRSEM and AFM enabled visualization of:

1. **Two steps of protein binding, showing the difference between specific and nonspecific binding.**
2. **DNA hybridization, distinguishing between binding of complementary and noncomplementary strands.**

The observations are in agreement with LSPR spectroscopy results.