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.

AFM

Protein-Protein Interactions

Preparation of a functionalized interface and protein immobilization

HRSEM

DNA Hybridization

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.