

Reference: John C. Sharpe *et al.* "Gold Nanohole Array Substrates as Immunobiosensors", *Analytical Chemistry*, 2008, 80, 2244-2249.

Bioanalysis and Sample: In this paper, the authors describe gold-nanohole-array surface plasmon resonance (SPR) biosensors to probe interactions under immunochemical conditions (antigen/antibody). A secondary antibody-gold nanoparticle conjugate is used to enhance the detection. This system is used to detect small molecules and has high sensitivity in a low affinity system. Cortisol is the model analyte in this paper. This regenerable system is also used for consistent multisample binding response.

Importance: As a surface plasmon resonance biosensor, the gold nanohole array system is a label-free and real-time analytical system. The device here takes advantage of the unique properties of nanohole arrays: surface-based sensitivity; transmission mode operation; a relatively small footprint; and repeatability. Cortisol is a vital hormone in humans which increases blood pressure, blood sugar levels and so on. This is the first report of nanohole arrays for detecting low affinity interaction under immunochemical condition. It shows a higher sensitivity in a regenerable format and has low nonspecific binding.

Technique: Surface plasmons (SPs) are surface-bound electromagnetic waves formed at the interface between a metal and a dielectric. The SPR condition can be achieved by either prism or grating coupling. Nanohole arrays belong to the second one. The tiny holes that are smaller than the wavelength of incident light can lead to many optical properties. The transmission at wavelengths that matches the SPR resonance condition is enhanced by the nanohole array. Peak wavelength red shift is used to denote binding responses for each interaction. The extent of SP generation depends on the combination of incident light wavelength, hole periodicity and material dielectric constants. In the paper, a white light source was used as the incident light and the transmission was measured over a wavelength range from 400 to 1100 nm. The cortisol-linker-thiol derivative is immobilized on a gold surface. The interaction between cortisol and monoclonal anti-cortisol is monitored by nanohole arrays. A secondary IgG-Gold nanoparticle conjugate was bonded to the first antibody which enhance the detection by 3 times. It provides possibility to produce portable and robust biosensors for detecting important biomolecules.

Example: The peak shift from monoclonal Ab (mAb) binding is about 2 nm which is less than 2.5 nm from secondary Ab binding and 7 nm from secondary Ab-Gold binding. It shows the secondary Ab-Au binding enhanced the signal by more than 3 times. So the sensitivity increases 3 fold. The peak shift for non-specific binding of secondary Ab-Au is less than peak shift for secondary Ab binding. Thus Ab-Au nanoparticle conjugate really enhances the detection and had low non-specific binding.

Opinion: This study showed a sensitive and regenerable nanohole array detection system under immunochemical conditions. The detection was enhanced 3-fold and non-specific binding was lowered by the second antibody-Au conjugate. It's better if the authors could show any data to verify this system is suited to inhibition assays.

