

## *Development of Plasmonics-Based Photoacoustic Immunoassay and a Photonic Crystal Substrate for Sensitivity Enhancement*

Immunosandwich assay is an ideal tool for targeted, high-throughput, and quantitative protein analysis. In such an assay, a pair of specific antibodies recognize the analyte, such as a disease biomarker, and the analyte concentration is measured using labels. Conventional immunoassays utilize fluorescent or chemiluminescence dye molecules or enzymes, i.e. enzyme-linked immunosorbent assay (ELISA), as the label to quantify the analyte concentration. By and large, fluorescence-based immunoassays are highly sensitive, but require expensive instrumentations, which limit its application in a point-of-care system in resource-limited settings. In addition, the fluorescent dyes are prone to photobleaching and affected by environmental conditions, which makes the quantitative analysis especially difficult. ELISA uses an inexpensive colorimetric detection system but it lacks the sensitivity to detect low-abundance biomarkers present at the early stage of diseases. To overcome these problems, we proposed a novel photoacoustic (PA) immunoassay using plasmonic nanoparticles to facilitate the detection of disease biomarkers. The principle of PA detection is based on the photothermal effect, in which the absorption of light by target substances generates heat. Illuminated by modulated light from a laser source, the samples will periodically generate heat, which causes the thermal expansion and contraction of the air in the vicinity. The expansion and contraction process results in a pressure wave, which is measured by a microphone. The advantages of the PA detection include a high sensitivity, a large dynamic range, and a low-cost instrumentation. The developed photoacoustic immunoassay uses gold nanoparticles (AuNPs) as a label that converts photons into acoustic waves and reports the analyte concentration. When the laser wavelength matches the absorbing center of the localized surface plasmon resonance, the collective oscillation of free electrons within AuNPs results in a significantly stronger photoacoustic conversion. The intensity of the acoustic wave generated by the AuNPs is correlated to the analyte concentration. The plasmonic-enhanced PA effect offers a highly sensitive and reliable approach for the detection of biomarkers. As an example, the PA assay of Human interleukin-8 was carried out using our PA detector and the results show a limit of detection of 1.6 pg/mL, which is one order of magnitude lower than the standard colorimetric assay.

To further improve the detection sensitivity, a nanostructured substrate was implemented to enhance the light absorption of the AuNPs. The substrate consists of a photonic crystal (PC) structure, which supports the guided-mode resonance (GMR), also known as the leaky mode resonance. When a laser light is coupled into a GMR mode, the resulting evanescent field near the PC surface is significantly enhanced compared to the intensity of the excitation beam. The enhanced near field allows the AuNPs, immobilized in the evanescent field region, to absorb more light and to consequently generate a stronger PA signal. It has been demonstrated that the PC substrate can improve the PA signal by a factor of 40 and enable the measurement of  $\sim 10$  AuNPs in an area of  $100 \mu\text{m}^2$ .