Electronic Supplementary Information

Paper-based SERS assay for sensitive and duplex cytokine detection towards atherosclerosis associated diseases diagnosis

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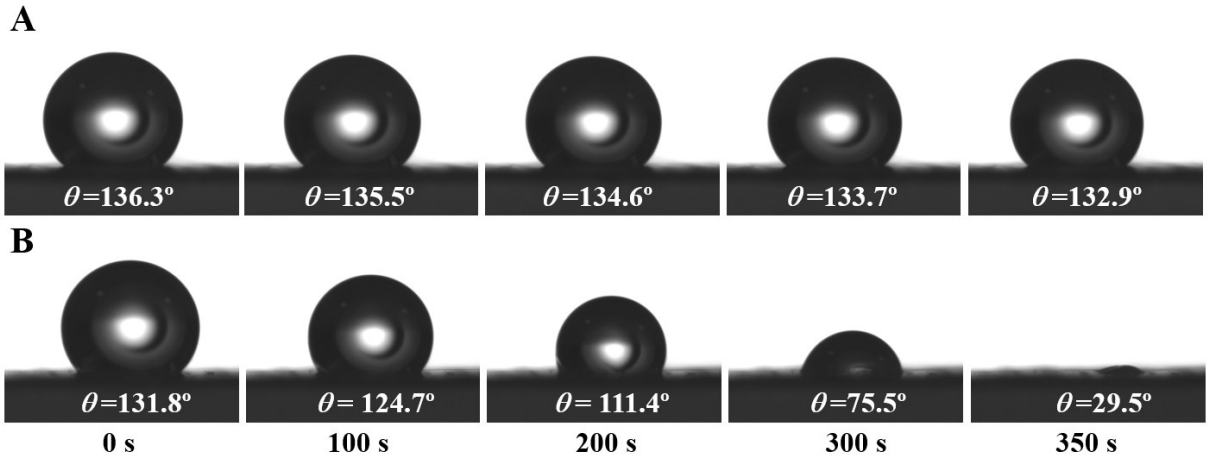

Fig. S1. Contact angle (θ) of 5 µL MilliQ water droplet on PTFE membrane surface (A) before and (B) after pre-treatment.

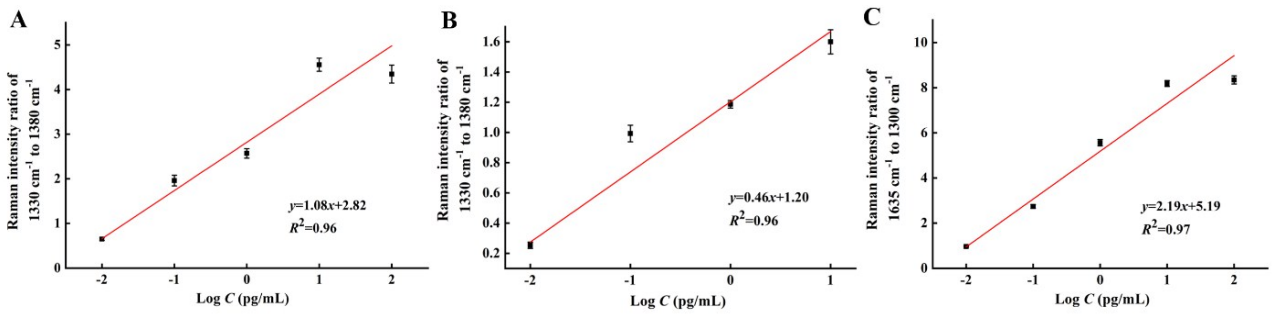

Fig. S2. Calibration curves between logarithmic concentration of target cytokines with SERS response. (A) IL-10 in PBS, (B) IL-10 in human serum and (C) MCP-1 in human serum.

Evaluation for the potential non-specific binding of SERS nanotags in deep layer of PTFE substrate

Since PTFE membrane is multi-layered, to investigate whether the non-specific binding of SERS nanotags in deeper membrane skeleton might affect the assay specificity towards target cytokines, the laser penetration depth (*d*) was evaluated. Basically, *d* was calculated with equation $d=\lambda/(NA)^2$,[1] where λ refers to laser wavelength, *NA* corresponds to numerical aperture of the used objective (long working distance, ×50). In this work, λ is 633 nm and *NA* is 0.6, giving a laser penetration depth of 1.76 μm. The thickness of PTFE membrane is 10 μm. Hence, we conclude that the SERS measurement only occurs at the top surface of substrate, the potential non-specific deeper binding of SERS nanotags will not affect the assay performance in specificity.

Reference

1 S. Laschi, I. Palchetti, and M. Mascini, *Sens. Actuators B: Chem.* 2006, **114**, 460-465.