Supplementary Material

Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip

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S1. Serological assays for anti-SARS-CoV-2 antibodies detection

Based on the most up-to-date survey of the existing commercial serological tests (U.S. Food and Drug Administration, 2020; Center for Systems Biology - Massachusetts General Hospital, 2020), Table S1 compares our opto-microfluidic sensing platform with those reported by FDA, highlighting the performance parameters such as the assay principle, target molecules, assay time, portability, user experience requirement, and susceptibility to false negative results. Standard serological assays such as enzyme linked immunosorbent assays (ELISA), chemiluminescent immunoassays, neutralization assays, and lateral flow assays are well established technologies, with their respective advantages and limitations. For example, ELISA and neutralization assays can deliver accurate results but are expensive, require trained operators, take longer assay time; while other assays are faster and cheaper but results are qualitative and less accurate (i.e., lateral flow assays). On the other hand, our platform can achieve the quantitative label-free detection of anti-SARS-CoV-2 antibodies with a relatively short assay time (i.e., ≈ 30 min). Based on Table S1, only Chemiluminescence Immunoassay (CLIA) and Electrochemiluminescence Immunoassay (ECLIA) offer comparable assay time (20–30 min) with quantitative information. However, our opto-microfluidic device is portable, label-free, cheaper and more user friendly.

We compare the limit of detection values (an important sensitivity parameter for biosensors) of our platform against those recently reported of commercial ELISA assays. Our device achieves the LOD of 0.08 ng/mL in 1:1000 diluted spiked human plasma sample (equivalently 80 ng/mL in non-diluted human plasma), which is comparable to the commercial ELISA kits (Zhang et al., 2014). For example, pGOLD$^{\text{TM}}$COVID-19 High Accuracy IgG/IgM Assay Kits (https://www.nirmidas.com/pgold-covid-19-igg-igm-assay-kit) report the LOD of 1.6 ng/mL for IgGs in human plasma. EDI$^{\text{TM}}$Novel Coronavirus COVID-19 ELISA Kits (https://www.epitopediagnostics.com/covid-19-elisa) report the LOD of 5 IU/mL (estimated to be $\sim$ 13500 ng/mL (Humphrey and Batty, 1974)) of IgGs against the nucleocapsid protein in human plasma. Since our platform does not require any labelling procedure, such as those involving fluorescence or chromatic changes which are common for ELISA assays, our device can be offered as a promising tool to complement existing COVID-19 serological assays. Bear in mind that our current platform is still at the proof-of-concept stage and our results are based on artificial human plasma samples doped with anti-SARS-CoV-19 S antibodies produced in rabbit.

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Table S1: Comparison with existing commercial COVID-19 serological assays.

Serology test	**Target**	**Assay time**	**Process type**	**FDA-EUA**	**Examples***	**Portability**	**Experienced operators**	**Susceptible to false negatives**
Our work	**IgG, IgM**	**∼30 min**	**LSPR opto-microfluidic device**	**N/A**	**N/A**	**Yes**	**No**	**No**
LFA	IgG, IgM	15 min	Cartridge	13	#Cellex, #Autobio, #ChemBio, #Healgen, Innovita, #Hangzhou Biotest Biotech, #Biohit, #Hangzhou Laihe Biotech, #BD, #Assure, others	Yes	No	Yes
ELISA	IgG, IgM	2-4 h	Plate	6	#Mount Sinai, #EUROIMMUN US Inc., #InBios, #Emory Medical, BioRAD, Snibe, Zhejiang Orient, Creative Dx, #InBios, #Beijing Wantai	No	Yes	No
CLIA	IgG, IgM	30 min	Cartridge	12	#Abbott, #DiaSorin, #Ortho-Clinical (2), #Vibrant America, #Siemens (4), #Babson Diagnostics, #Diazyme, #Beckman	No	Yes	No
EIA	IgG, IgM	2 h	Plate	2	#BioRAD, #BioMérieux	No	Yes	No
MIA	IgG, IgM	N/A	Plate	4	#Wadsworth Center, #Siemens (2), #Luminex	No	Yes	No
ECLIA	IgG, IgM	20 min	Plate	2	#Roche (2)	No	Yes	No
ECS	IgG, cytokine	1 h	Cartridge	0	#Accure Health	Yes	No	N/A
Microarrays	Ig epitopes	1.5 h	Plate	0	RayBiotech, PEPperPRINT	No	Yes	No

Legend
* EUA Authorized Serology Test Performance from FDA (accessed: 2020-08-15)
# FDA-EUA cleared tests commercially available

LFA - Lateral flow assay
ELISA - Enzyme Linked Immunoabsorbent Assay
CLIA - Chemiluminescence Immunoassay
EIA - Enzyme Immunoassay
ECLIA - Electrochemiluminescence Immunoassay
ECS - Electrochemical Sensing

S2. Cyclic voltammetry and chronoamperometric measurements

The cyclic voltammogram of the electrolyte solution (8 mM of $AuCl_3$ and 0.5 mM of $Pb(CH_3COO)_2$) used to fabricate the gold nanospikes on the electrode surface is shown in Fig. S1a.

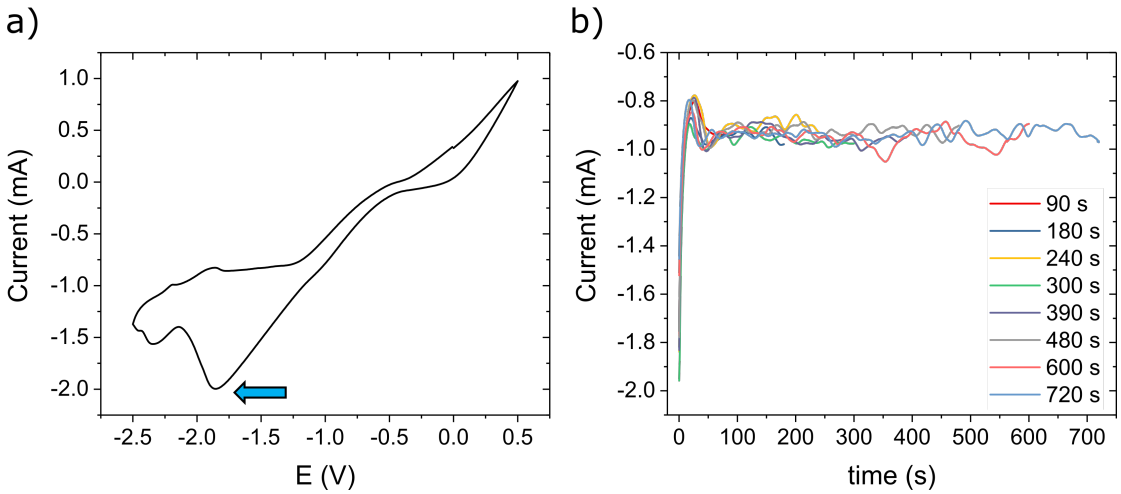

Figure S1: (a) Cyclic voltammogram of the electrolyte solution used for the gold electrodeposition containing 8 mM of $AuCl_3$ and 0.5 mM of $Pb(CH_3COO)_2$ at 100 mV/s. (b) Chronoamperograms recorded during the formation of gold nanospikes with different sizes. The applied voltage is -1.85 V.

As reported in literature (Plowman et al. (2009); Sabri et al. (2014)), the presence of lead ions drives the formation and the growth of the gold nanospikes without affecting their surface chemistry. The electrodeposition (ED) process is performed at a constant potential of -1.85 V for 90, 180, 240, 300, 390, 480, 600 and 720 s. The corresponding current profiles are reported in Fig. S1b. The current intensity rapidly increases and stabilizes after ED time of $\approx$ 50 s at $\approx$ 0.9 mA with fluctuations of $\approx$ 50 μA.

S3. Morphological, optical and structural characterization of the gold nanospikes

The gold nanospikes fabricated at different ED times are characterized by scanning electron microscopy (SEM), UV-Vis spectroscopy, and X-ray Diffraction (XRD).

The SEM images (Fig. S2) show that the surface roughness increases with the increasing ED time (up until 240 s). At 300 s of ED time, small gold nanostructures start to emerge. These structures become bigger with increasing ED times. Under ED time of 480 s, the gold nanospikes have the most regular morphology and are homogeneous across the substrate. By further increasing the ED times (i.e., 600 s and 720 s), the nanostructures become more heterogeneous.

When the ED times are varied from 90 s to 480 s, the increase in the average size of the gold nanostructures with the increasing ED time yields an increase in the absorbed light (Fig. S3a), thus a progressive red-shift in the absorption maximum is observed (Fig. S3b). However, when further increasing the ED time (i.e., 600 s and 720 s), the heterogeneous nature of the gold nanostructures induces more noise in the absorption spectra, leading to a blue shift in the resonance peak.

XRD $2\theta - \omega$ scans (scanning of the incident and diffracted beam) are carried out with a Bruker D8 Discover diffractometer (Bruker, Japan) equipped with a copper line focus tube and a Göbel mirror that produce a beam of Cu Kα radiation of 0.15418 nm average wavelength. On the primary beam side, a 1.2 mm divergence slit is used. On the secondary beam path, a fully open detector slit and a 0.2° Soller slit are employed in front of a scintillation counter detector. First, XRD is carried out on a bare gold coated glass slide as a reference. Its diffraction pattern (red curves of Fig. S4a and b) reveals the presence of a pronounced (111)-texture in the Au film. Indeed, the ratio of the intensities of the (111) and the (200) Bragg peaks is $\sim$100 versus $\sim$2 which is expected for Au powders. The gold nanospike covered substrate (produced after 480 s of ED) exhibits more intense (111) and (222) Bragg peaks (blue curve in Fig. S4a), indicating that the gold nanospikes mostly grow (111)-oriented direction perpendicular to the surface (red curve). On the

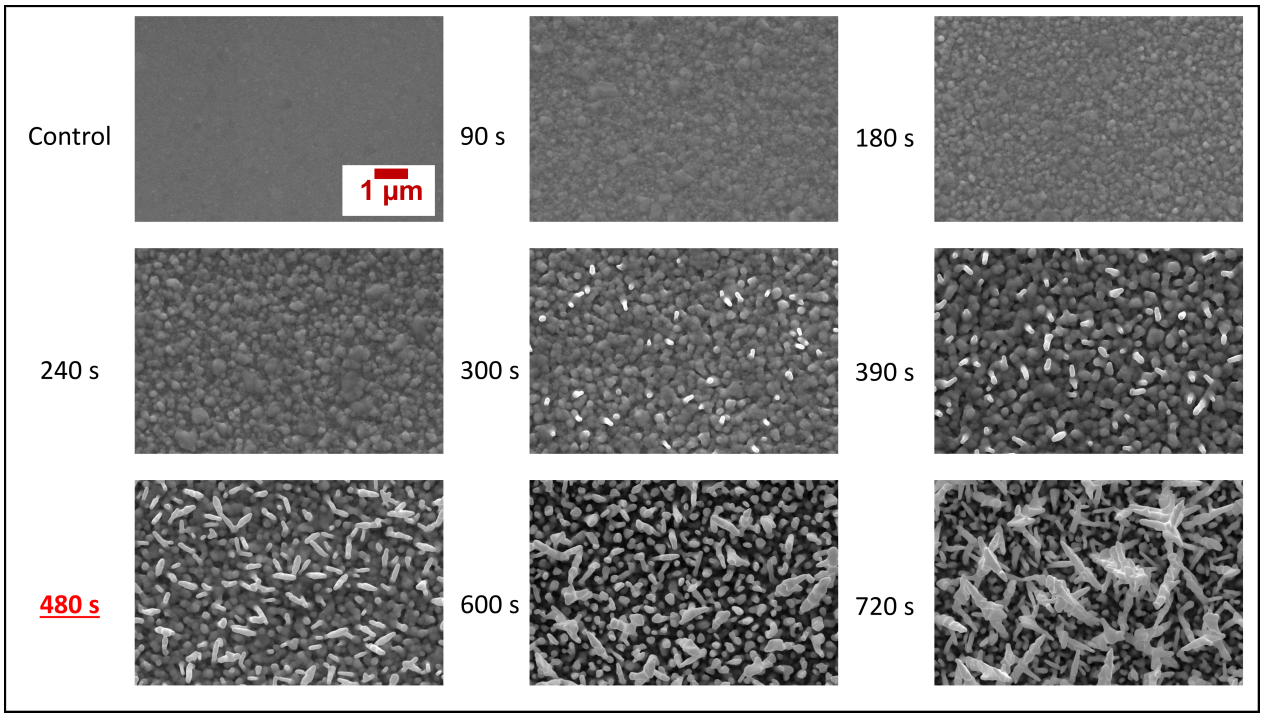

Figure S2: SEM images of the gold electrode treated by electrodeposition. The ED time of 480 s delivers the most homogeneous gold nanospiked structures.

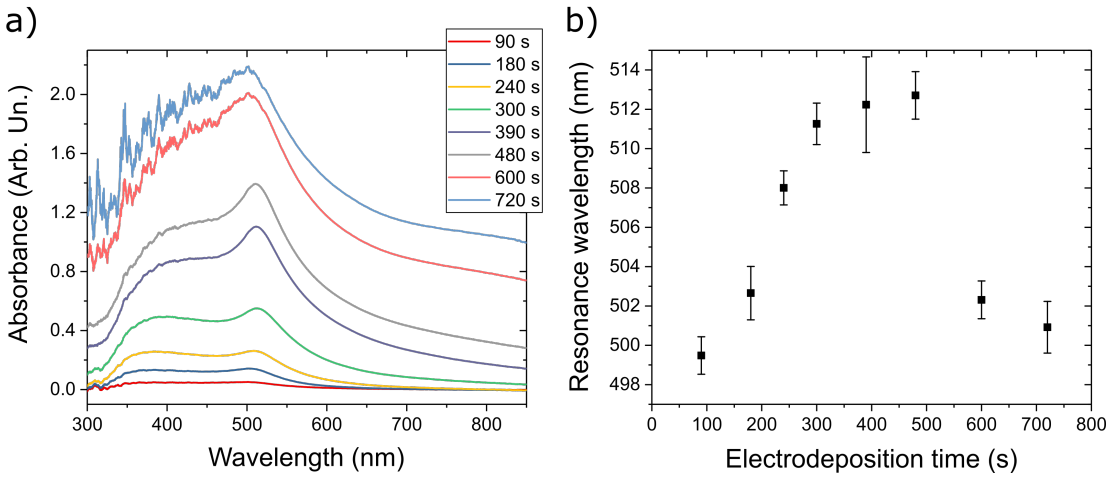

Figure S3: Optical properties of the gold nanostructures. (a) Absorbance spectra and (b) resonance maxima for samples produced at different electrodeposition (ED) times.

other hand, for gold nanostructures produced under the longest ED time (720 s), an increase of intensity of nearly all the peaks (green curve in Fig. S4b) is observed with respect to the reference. Furthermore, in the 720 s specimen, the (111) Bragg peak and its second order, the (222) reflection, are less intense than those in the 480 s sample. This suggests an increase of heterogeneity in the nanostructures produced at 720 s. These XRD results are consistent with the SEM images shown in Fig. S2, i.e., for ED time of 720 s, the gold nanostructures become more disordered, with dendrites formed from the original nanospike stem.

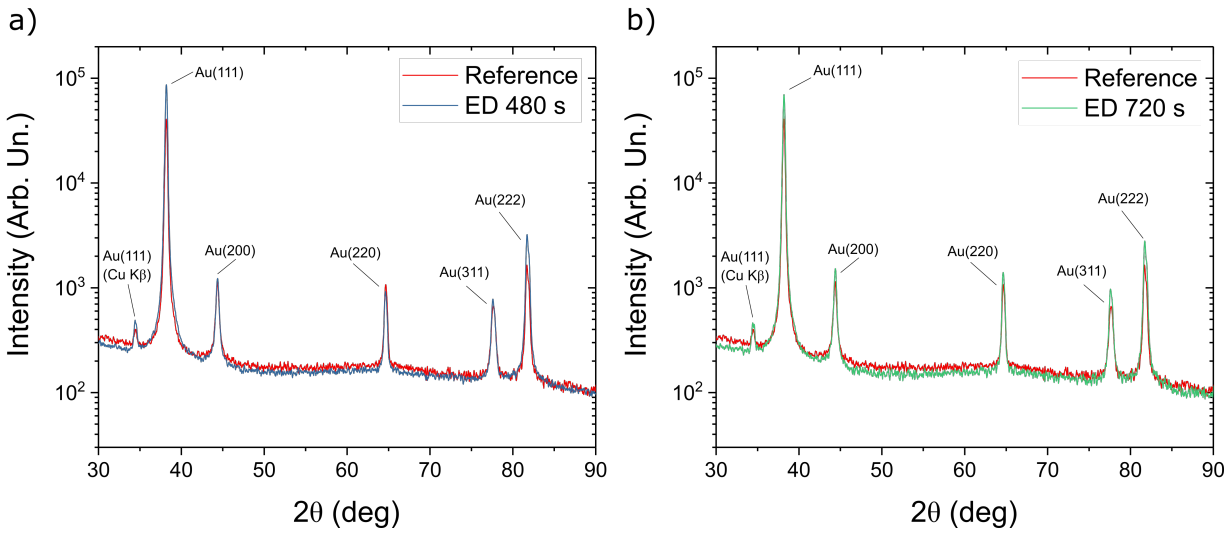

Figure S4: XRD 2θ-ω scans of gold coated glass slide, with 480 s and 720 s electrodeposition time. (a) Scans of the bare gold coated glass slide as control (red curve) and gold nanospikes produced after 480 s of ED time (blue curve). (b) Scans of the bare gold coated glass slide as control (red curve) and gold nanostructures produced after 720 s of ED time (green curve). The scans are taken across the full width 2θ range (20–125 deg), covering Au(400), Au(331) and Au(420) peaks. A shorter range (30–90 deg) is displayed to highlight the differences in peak intensities. The peak behavior in the range which is not shown does not alter the conclusions. The weak reflection at the lowest 2θ angle is caused by stray Cu Kβ radiation diffracting from Au(111) netplanes.

S4. Functionalization of gold nanospike covered substrate validated by LSPR

Each step during the functionalization of gold nanospike covered substrate in air is validated by measuring the wavelength shift of the LSPR peak position on the substrate (Fig. S5).

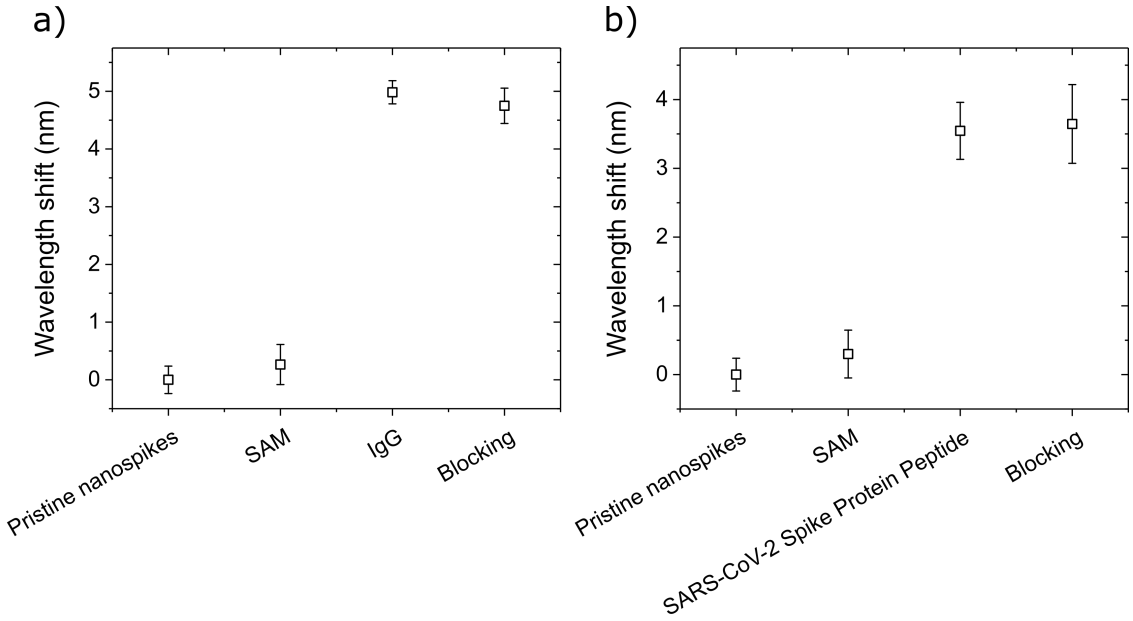

Figure S5: LSPR peak position at each functionalization step for (a) the IgG/anti-IgG model system, and (b) the SARS-CoV-2 Spike protein peptide and anti-SARS-CoV-2 Spike protein antibody system.

The spectra are recorded after washing the gold nanospike covered substrate 3 times with MilliQ water, followed by drying with a gentle stream of nitrogen. Starting from the pristine gold nanospikes, the self-

assembled monolayer (SAM) of alkyl thiols produces a small red shift of ≈ 0.3 nm. This is followed by the immobilization of the antigen (1 μg/mL) which produces a much larger wavelength shift in the peak position (i.e., 5 nm for IgG and 3.5 nm for the SARS-CoV-2 Spike protein peptide). The wavelength shift difference between these two cases is caused by the different size of the 2 antigen molecules. After immobilizing the antigen, the substrate is passivated with a blocking agent bovine serum albumin (BSA) at 100 μg/mL. This step produces a negligible change in the LSPR peak position, verifying that the gold nanospike covered substrate is effectively functionalized by the antigen molecules.

References

U.S. Food and Drug Administration, *EUA Authorized Serology Test Performance* (2020), accessed: 2020-08-15, URL `https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance`.

Center for Systems Biology - Massachusetts General Hospital, *COVID-19 Diagnostics In Context* (2020), accessed: 2020-08-15, URL `https://csb.mgh.harvard.edu/`.

S. Zhang, A. Garcia-D'Angeli, J. P. Brennan, and Q. Huo, Analyst **139**, 439 (2014).

J. Humphrey and I. Batty, Clinical and Experimental Immunology **17**, 708 (1974).

B. Plowman, S. J. Ippolito, V. Bansal, Y. M. Sabri, A. P. O'Mullane, and S. K. Bhargava, Chemical Communications **33**, 5039 (2009).

Y. M. Sabri, S. J. Ippolito, J. Tardio, V. Bansal, A. P. O'Mullane, and S. K. Bhargava, Scientific Reports **4**, 1 (2014).