Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Amplification-free SERS Analysis of DNA Mutation in Cancer Cells with Single-base Sensitivity

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1. Schematic illustration of the sensor fabrication process

Step 1: The glass slide was modified with thiol group through the MPTMS treatment.

Step 2: To immobilize the nanoparticles onto the desired region in a rectangle shape, a drilled PDMS piece (not plotted in the figure) was used as a frame template to confine the nanoparticle solutions. Here, the PDMS frame was reversibly bonded against the thiol-modified glass slide. Then the Au@Ag NRs colloid solution was added to the PDMS template, so that the Au@Ag NRs were immobilized onto the glass slide in the confined rectangle region through the Ag-S bond.

Step 3-4: After washing, the molecular beacon probes were subsequently added to the template, so that the thiol-modified probes were attached to the nanorods through the Ag-S bond. The PDMS frame was then peeled off from the glass slide, while the molecular beacon-attached nanorods remained on the glass slide in a rectangle shape.

Step 5: The PDMS piece containing the microchannel was treated with oxygen plasma and then irreversibly bonded against the untreated glass slide previously modified with a strip of nanorods and probes, in the orthogonal direction.

Step 6: DNA hybridization in the microchannels.

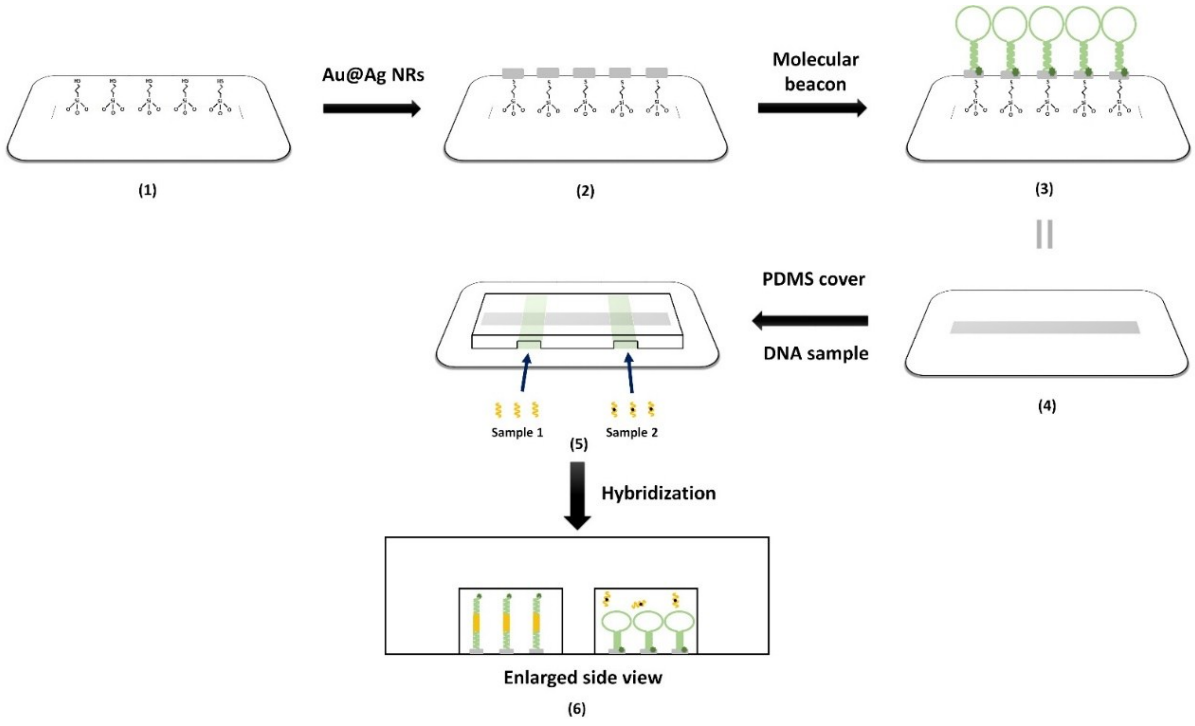

Figure S1. Development of the SERS-based DNA assay on a microfluidic chip. (1) The glass slide was modified with MPTMS. (2) Au@Ag NRs were immobilized on the glass slide. (3) Molecular beacon probe was attached to the surface of the nanoparticles. (4) Simplified description of (3), also applicable to Fig.1. (5) The glass slide was bonded against a PDMS piece containing the microchannels, through which the samples were flowed. (6) Enlarged side view of the microfluidic channel after hybridization.

2. Sequences of nucleic acid chains (from 5' to 3' end)

Molecular beacon:

FAM CGCGC GTTGGAGCTGGTGGCGTAG GCGCG $(CH_2)_6$ -SH

(FAM is short for carboxyfluorescein)

Wild-type KRAS: CTACGCCACCAGCTCCAAC

Mutant KRAS (G12V): CTACGCCAACAGCTCCAAC

3. Calculation of the SERS enhancement factor (EF)

The characterization of SERS enhancement factor (EF) was performed according the method by Mao *et al.*[1]

The Raman reporter (DTNB) was used to test the EF of the prepared SERS-active substrate. In the experiment, the same volume (1 μL) of DTNB solution in concentrations of 1 μM and 100 mM was added to the SERS substrate and the bare glass slide respectively. After the evaporation and drying process, the DTNB molecules remained on the surfaces, covering a surface area of around $\pi \times 2^2$ mm^2.

The SERS EF was calculated according to the following equation:

$$EF = (I_{SERS} / N_{SERS}) / (I_{Raman} / N_{Raman}) \quad (1)$$

where I_{SERS} and I_{Raman} are the Raman intensities of DTNB on the SERS substrate and glass slide respectively. N_{SERS} and N_{Raman} are the number of molecules in the scattering volume.

As the size of the laser spot (around 1 μm) is the same for both substrates, the ratio of molecule counts in the scattering volume is approximately equal to the ratio of the DTNB concentration. Thus, the ratio of N_{SERS}/N_{Raman} is approximately equal to 100 mM / 1 μM = 10^5. According to the measurement results, the ratio of average SERS intensity (I_{SERS}/I_{Raman}) is 18.5, so the enhancement factor is estimated to be 1.85×10^6. Using this method, there is no need to estimate the exact amount of molecules in the scattering volume.

We can also estimate the number of molecules in the scattering volume. Assuming that the DTNB molecules are uniformly distributed and considering that the laser spot is around 1 μm in diameter. The number of molecules in the scattering volume is estimated to be

$$N_{SERS} = 1\ \mu L \times 1\ \mu M \times N_A \times (\pi \times 0.5\mu m \times 0.5\mu m)/(\pi \times 2mm \times 2mm) = 3.8 \times 10^4$$

$$N_{Raman} = 1\ \mu L \times 100\ mM \times N_A \times (\pi \times 0.5\mu m \times 0.5\mu m)/(\pi \times 2mm \times 2mm) = 3.8 \times 10^9$$

4. Characterization of the stability of the SERS substrate over time

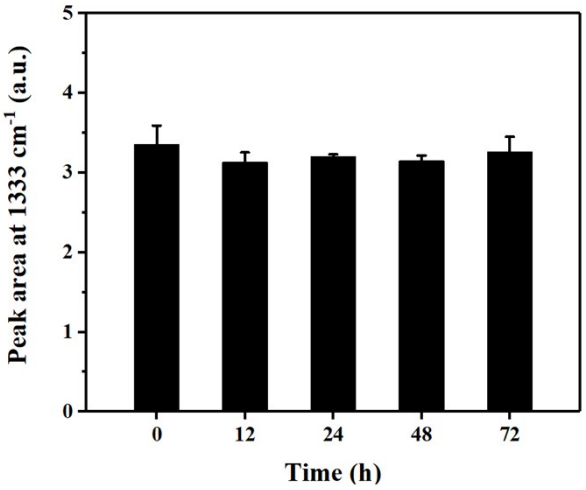

Figure S2. Stability test of the SERS substrate. Peak area at 1333 cm^{-1} of DTNB molecules measured at different time after the fabrication of SERS substrate.

5. Calculation of the signal-to-noise ratio of the SERS spectra

The signal-to-noise ratio (S/N) was calculated with the SERS spectrum of a Raman the molecular beacon probe which contains a Raman molecule (FAM). As shown in Fig. S3, the peak signal is measured at 1325 cm^{-1}, while the noise was measured at the 1075 cm^{-1} where there should be no Raman peaks. The measurement provided the following data:

Peak signal at 1325 cm^{-1} = 17018

Background at 1075 cm^{-1}= 12041

The S/N is calculated to be

$(17018-12041) / (12041)^{1/2} = 45.4$

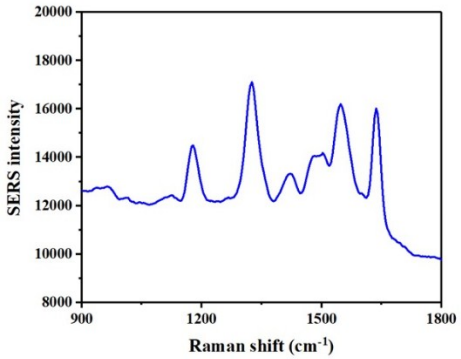

Figure S3. SERS spectra of the molecular beacon on SERS substrate.

6. Characterization of the size of DNAs extracted using the Purelink DNA extraction kit.

The DNA extraction kit selected for obtaining the genomic DNA from the cell lines in this study (Purelink Genomic DNA kits, Invitrogen) specifies that the final genetic material should be in the range of 20k bp to 50k bp.

Here, a gel electrophoresis has been performed to confirm the optimal performance of the kit. As can be observed in Fig. S4, genomic DNA larger than 1500 bp was obtained from 3 different cell lines (HT-29, MDA-MB-435 and SW480), including the 2 selected for this work (MDA-MB-435 and SW480).

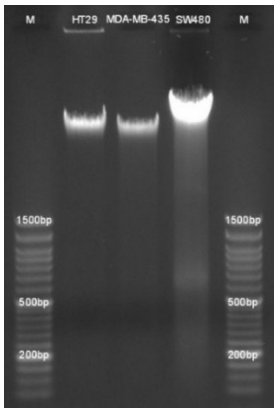

Figure S4. Gel electrophoresis results of the DNA extractions from HT29, MDA-MB-435 and SW480 cells. The bands on the left and right (labelled with 'M') corresponds to the model nucleic acids for reference.

7. Calculation of surface concentration of the hairpin DNA on SERS substrate

The NanoDrop Spectrophotometer has been used to characterize the concentration of DNA by measuring the absorption at 260 nm (A260), which corresponds to the maximum absorption of nucleic acids. As detailed in the experimental section, 100 μL of 0.5 μM hairpin DNA solution was added onto the SERS substrate with an area of around 3 mm×18 mm. The A260 values of supernatant before and after DNA immobilization were 0.190 and 0.172 respectively. Therefore, the surface concentration is estimated to be

$$(0.5\ \mu M \times 100\ \mu L \times (0.190\text{-}0.172)/0.190) / (3mm \times 18\ mm) = 88\ fmol/mm^2 \quad (2)$$

As the width of the channel was 600 μm, the amount of hairpin DNA in the each channel is estimated to be

$$88\ fmol/mm^2 \times (3mm \times 600\ \mu m) = 158\ fmol \quad (3)$$

The maximum concentration of samples used in this experiment was 5 nM, and the volume was 25 μL. Hence, the corresponding amount of target was approximately

$5\ nM \times 25\ \mu L = 125\ fmol,$ (4)

which is smaller than 158 fmol.

Therefore, there should be enough molecular beacons to hybridize the target for SERS detection.

On the other hand, we also calculated the surface density of a packed monolayer of hairpin DNAs. Considering the hairpin structure and the length of a single base (0.34 nm), the width of one hairpin DNA is approximately 2 nm. Supposing that the hairpin structures are closely packed, the maximum surface density is estimated to be

$1/(0.34\ nm \times 2\ nm) = 2500\ fmol/mm^2,$ (5)

However, when opening, the molecular beacon should occupy about (0.34nm×24 nucleotides), so to allow opening, maximum surface density should be $208\ fmol/mm^2,$

which is larger than 88 fmol/mm^2.

Hence, there should also be enough free space for the molecular bacons to hybridize with the target sequences.

8. Schematic illustrate of the regions for acquiring SERS spectra

As shown in the photo of the chip in Fig.S5a, the microfluidic chip contains 8 parallel straight channels. The SERS substrate is embedded below the channels. Different samples were flowed through different channels respectively. The hybridization happens in the 8 cross regions between the channels and the SERS substrate. After reaction, the SERS spectrum were acquired in these cross regions. In those regions without SERS substrate, there is definitely no SERS signal. Fig.S5b shows the regions for SERS acquisition. Each SERS detection result in this manuscript was obtained from the SERS region in each corresponding channel, in which the whole microfluidic networks contains 8 independent SERS detection results of 8 different samples.

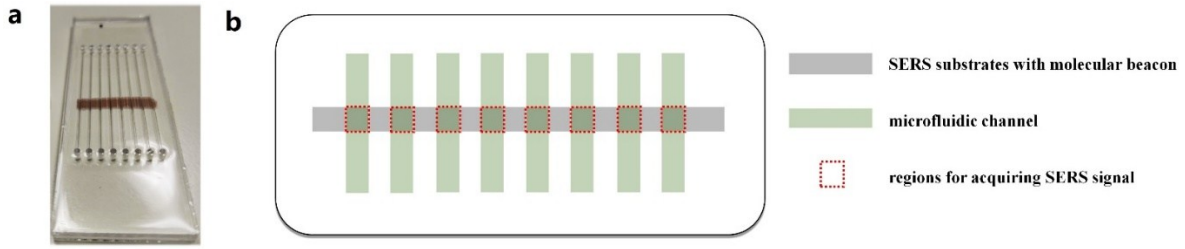

Figure S5. (a) Photo of the real chip (b) Schematic illustration of the regions for SERS signal acquisition (top view). The signal measured in each region corresponds to the sample flowed through its corresponding channel.

9. Characterization of Au@Ag NRs with high resolution TEM imaging

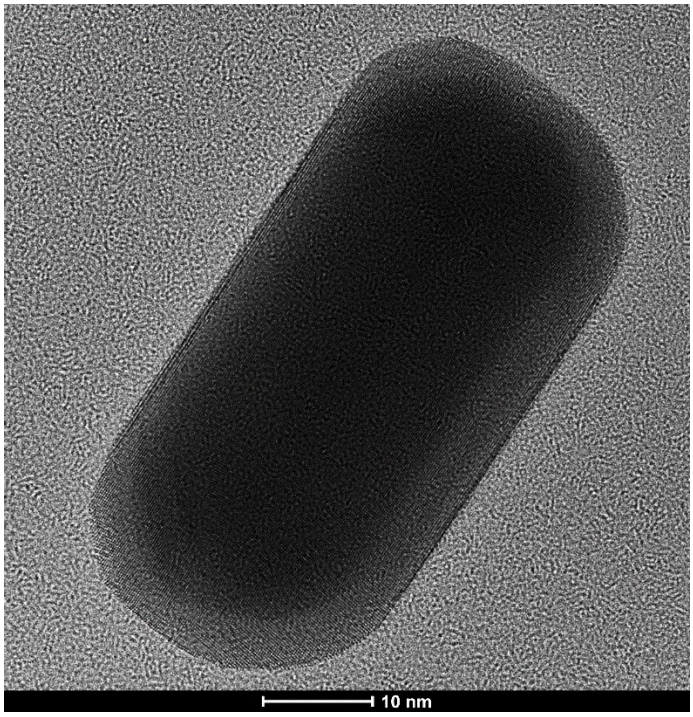

Figure S6. High resolution TEM image of Au@Ag NRs

10. Identifying the mutation status of cellular DNA samples with real-time PCR

Real-time PCR has been employed to analyse the cellular DNA to confirm the results of SERS assay. Cellular DNA samples with the same content as used for SERS detection have been tested with real-time PCR. As shown in Fig., the cycle quantification (Cq) value is negatively correlated with the percentage of mutation in positive samples (wild-type cellular DNA: mutant cellular DNA = 9:1, 5:5, 1:9, and 0:10, which correspond to 10%, 50%, 90% and 100% of mutation). Wild-type cellular DNA sample (wild-type cellular DNA: mutant cellular DNA =10:0, i.e. 0% of mutation) and no template control (NTC) were identified as negative. The SERS assay result is in accordance with the real-time PCR results, which further confirmed the ability of this sensor for cellular mutation analysis.

Protocols for real-time PCR:

The TaqMan® Mutation Detection Assay was performed to confirm the mutational status of cellular DNA samples. The reactions were performed in a final volume of 20 μL composed of 10 μL of Master mix, 2 μL of KRAS_515_mu probe (Thermo Fisher) and 2 μL of DNA

template, while the remaining volume corresponded to milli-Q Water. The following thermal profile was selected. 10 min at 95 °C for polymerase activation, followed by 5 cycles at 92 °C for 15 s and 58 °C for 1 min; and finally, another 40 cycles of 92 °C for 15 s and 60 °C for 1 min.

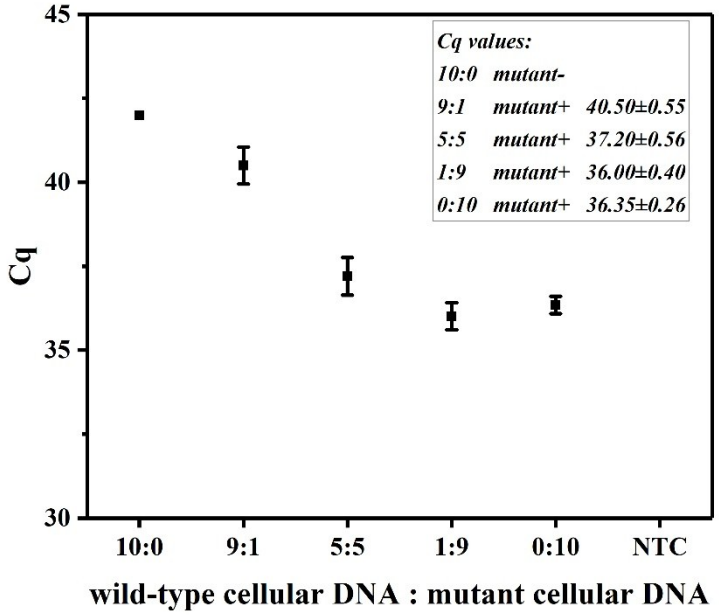

Figure S7. Detection of KRAS mutation in two cancer cell lines using real-time PCR. DNAs extracted from MDA-MB-435 and SW480 cell lines were mixed in ratios of 10:0, 9:1, 5:5, 1:9 and 0:10. The concentration of total DNA in each sample was 40 μg/mL. NTC represent the no template control. The error bars represent the standard deviation for four measurements.

11. Fluorescence assay with the SERS substrate

Fluorescence images have been taken to confirm the status of the probe. When the molecular beacon formed a hairpin structure, the FAM molecules is close to the substrate, there should be fluorescence quenching (fluorescence off). After hybridization with target DNA, the transformation of hairpin DNA would make the FAM molecule moving away from the substrate, during which the fluorescence should recover (fluorescence on). In the experiment, 5nM target DNA and the blank sample were tested. As shown in Fig.S8, the fluorescence intensity measured with target DNA was stronger than that tested with the blank sample, which confirmed structure transformation of the molecular beacon during target hybridization.

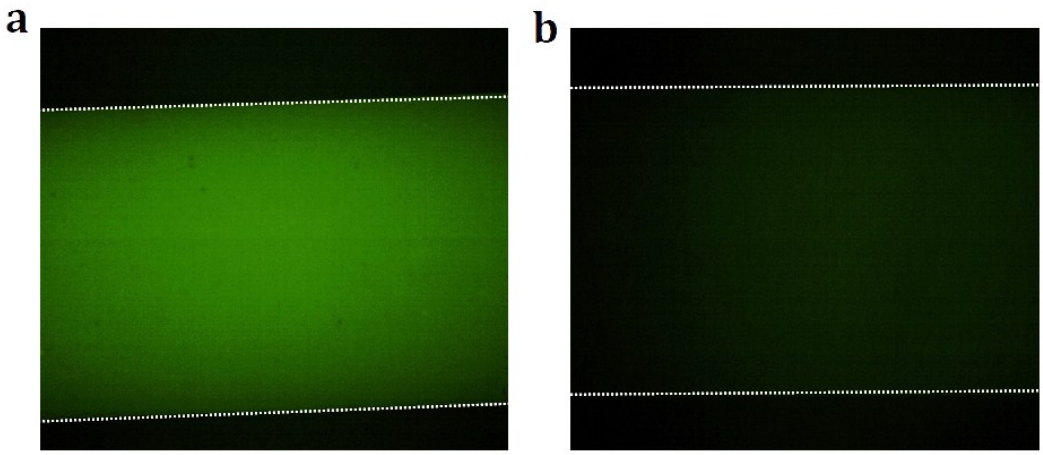

Figure S8. Fluorescence imaging of the DNA assay with 5nM target DNA (a) and the blank control (b). The scale bars indicate a length of 100 μm. The channels are profiled by the dotted lines.

Reference

1 H. Mao, W. Wu, D. She, G. Sun, P. Lv and J. Xu, *Small*, 2014, **10**, 127–134.