Label-free, *in situ* SERS monitoring of individual DNA hybridization in microfluidics

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Nanoporous gold (NPG) disks

The NPG disks were fabricated using a combined top-down and bottom-up approach. The initial film stack, consisting of a 75 nm thick Au:Ag=28:72 alloy film over a 300 nm thick base layer of Au, was deposited by DC sputtering. The gold target was a 99.99 % pure, Maple Leaf coin (Royal Canadian Mint); the alloy target was provided by ACI Alloys. The deposition rates for the gold and alloy films were 37.5 nm/min and 25 nm/min, respectively. The stack was patterned by RF-sputter-etching in 99.999% argon gas through a drop-coated mask of 500 nm polystyrene (PS) beads. RF-etching was timed to produce completely isolated alloy disks each sitting on a 65 nm thick solid gold pedestal; the remaining gold film provides a ground plane about 235 nm thick. The PS spheres were removed by sonication in isopropanol for 30 s. Ag was selectively dissolved by dipping in 70% room temperature HNO_3 followed by deionized water rinse and nitrogen dry to form the NPG disks. The entire dipping-transfer procedure takes ~5 sec. The resulting NPG disks are shown in Fig. S1(a). Benzenethiol molecules were employed to characterize the enhancement factor(EF) since they can form self-assembled monolayer on gold surface. NPGDs was soaked in 5nM benzenethiol solution for 30 min and rinsed in ethanol for 1 min. Fig. S1(b) shows the average SERS spectrum from a single NPG disk. The EF is calculated to be ~ 5×10^8.

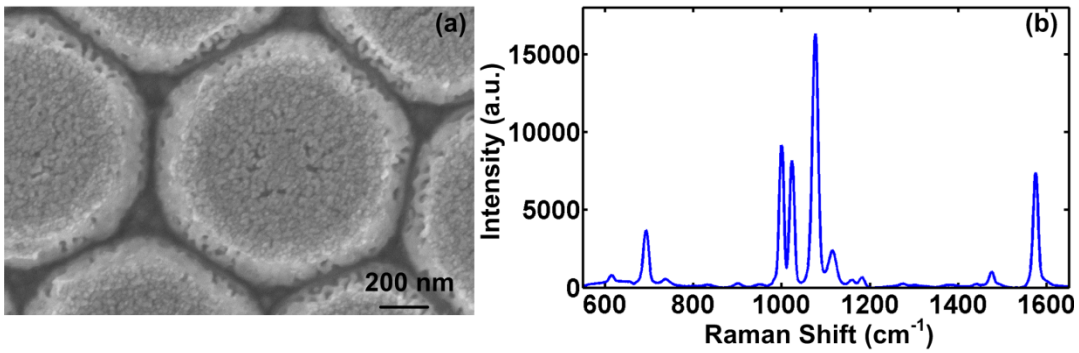

Figure. S1. (a) Scanning electron micrograph of NPG disks; (b) average SERS spectrum from a single NPG disk.

Probe density estimation

The average surface density of MS probe was estimated based on the measured spot area from drop cast and the volume and concentration of the MS probe solution. SERS intensity of Cy3 was used to characterize the number of probe molecules on the surface. For example, five SERS measurements were taken near the center of the dried spot by 2 μL 100 pM MS probe solution. This was to avoid taking data from the circumferences where "coffee ring" effect is apparent. The average SERS spectra are shown in

Fig. S2(a). The round shaped area was ~ 3mm diameter, resulting in a surface density of 42.6 molecules/μm^2 (blue spectrum). We observed a ~ 80% intensity decrease after MCH rinse (red spectrum), suggesting the probe density was 8.5 molecules/μm^2. An additional 50% intensity drop was observed after the following DI water rinse, leading to 4.2 molecules (black spectrum). Considering the surface coverage of the NPG disks to be ~50%, the average probe density on NPG disks is about 2 molecules/μm^2. We note that this represents a conservative estimate (i.e. upper bound) because we intentionally avoided the circumferences where more molecules accumulated.

We also studied the probe density distribution over the entire dried spot. Four SERS measurements were performed at the center, halfway and circumference of the dried spot, respectively. Figure S2(b) shows the Cy3 intensities at different positions just after the final rinse. The 12 red dots and the red circle schematically in the lower right corner represent measurement positions with respect to the dried spot. Cy3 intensities were lower at the center and higher at the edge. This again suggests our probe density estimate likely represents an upper bound. The probe density on NPG disk substrates using the incubation method was estimated by comparing the SERS intensity with the drop cast method. As shown in Fig. 1(b), the average SERS intensity from substrates incubated in 1 nM probe solution was similar to substrates using drop cast. Thus we concluded that the probe density was about 2 molecules/μm^2 for NPG disk substrates incubated in 1 nM probe solution. Similarly, the probe density for NPG disk substrates incubated in 5 nM probe solution was estimated to be about 10 molecules/μm^2.

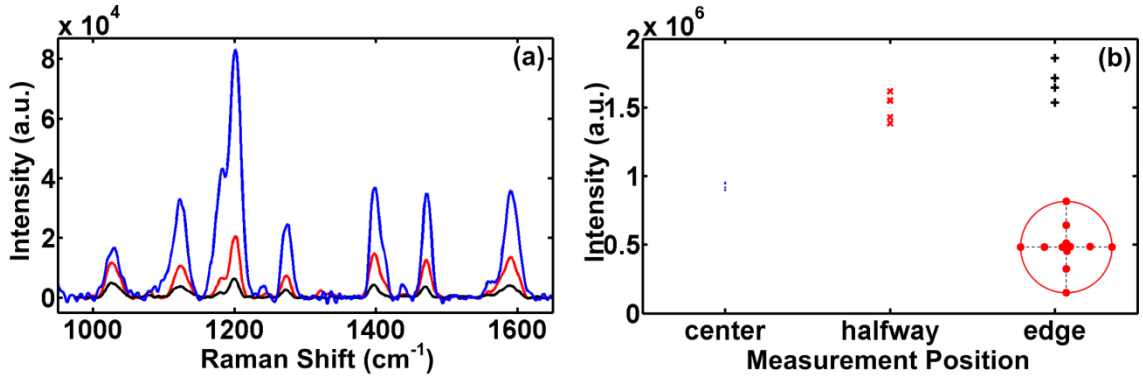

Figure. S2. (a) averaged SERS spectra before (blue), after (red) MCH treatment and after buffer wash step (black); (b) Cy3 SERS intensities at different physical positions, the red circle represents the dried area of probe solution, and the red dots represent measurement positions.

DNA hybridization temperature

We employed two different temperature for DNA hybridization experiments. For experiments using incubation at 5 nM for MS probe immobilization, 37.5°C was used. For the experiment using incubation at 1 nM for MS probe immobilization and the one with drop cast immobilization, 50°C was used.