Comparison of Fe_2O_3 and Fe_2CoO_4 core-shell plasmonic nanoparticles for aptamer mediated SERS assays

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ABSTRACT

Conjugation of oligonucleotides or aptamers and their corresponding analytes onto plasmonic nanoparticles mediates the formation of nanoparticle assemblies: molecularly bound bundles of nanoparticles which cause a measurable change in the colloid's optical properties. Here, we present further optimization of a "SERS off" competitive binding assay utilizing plasmonic and magnetic nanoparticles for the detection of the toxin bisphenol A (BPA). The assay involves 1) a 'target' silver nanoparticle functionalized with a Raman reporter dye and PEGylated BPA-binding DNA aptamers, and 2) a version of the toxin BPA, bisphenol A diglycidyl ether (BADGE), PEGylated and immobilized onto a silver coated magnetic 'probe' nanoparticle. When mixed, these target and probe nanoparticles cluster into magnetic dimers and trimers and an enhancement in their SERS spectra is observed. Upon introduction of free BPA in its native form, target AgNPs are competitively freed; reversing the nanoparticle assembly and causing the SERS signal to "turn-off" and decrease in response to the competitive binding event. The assay particles were housed inside two types of optofluidic chips containing magnetically active nickel pads, in either a straight or spotted pattern, and both Fe_2O_3 and Fe_2CoO_4 were compared as magnetic cores for the silver coated probe nanoparticle. We found that the Ag@ Fe_2O_3 particles were, on average, more uniform in size and more stable than Ag@ Fe_2CoO_4, while the addition of cobalt significantly improved the collection time of particles within the magnetic chips. Using 3D Raman mapping, we found that the straight channel design with the Ag@ Fe_2O_3 particles provided the most uniform nanoparticle organization, while the spotted channel design with Ag@ Fe_2CoO_4 demonstrated a larger SERS enhancement, and thus a lower limit of detection.

Keywords: Surface enhanced Raman spectroscopy (SERS), aptamer, plasmonic nanoparticles, competitive binding assay, molecular diagnostics

1. INTRODUCTION

While a number of nanoparticle based assays exist and have been exhaustively studied, these techniques have not been translated to a commercially viable diagnostic technology. This could be due to difficulties translating traditional enzyme-based assays into a robust lab-on-a-chip device due to issues with sample preparation and care, or because the persons running the test requires some degree of laboratory training and skill. We offer a solution to this problem through the utilization of aptamers, high affinity ligands synthesized from short (<20 basepairs) ssDNA folded into a tertiary structure, immobilized onto a combination of plasmonic and magnetic nanoparticles, and housed in a convenient magneto-optofluidic chip. By controlling and optimizing the magnetic and plasmonic nanoparticle aggregation state and dissociation response to an aptamer's analyte using a lab-on-a-chip platform, we have the ability to provide localized areas of concentrated sensing particles, allowing for more repeatable spectral analyses.

Optical properties of colloidal nanoparticles can be manipulated, tuned, and monitored for plasmonic sensing applications by forcing labeled particles into a controlled, but reversible, aggregative state.[1-3] Due to the versatility of DNA aptamers, aptamer-mediated surface enhanced Raman spectroscopy (SERS) has recently emerged as an attractive translatable platform method for biomarker detection of virtually any biomarker, ranging from bacterial, fungal, and viral targets to neurotransmitters, peptides, and proteins.[4,5] Conjugation of aptamers and their corresponding analytes onto plasmonic nanoparticles labelled with a Raman reporter molecule (RRM) mediates the formation of nanoparticle assemblies: molecularly bound bundles of stable colloidal nanoparticles which cause a measurable change in the colloid's SERS intensity.[1-3] To improve and simplify the detection of the small toxic molecule bisphenol A by its aptamer, multiple plasmonic and magnetic nanoparticle combinations were investigated and the SERS assay response in both a traditional liquid suspension and housed within a microfluidic chip are analyzed and compared.

Herein we present the optimization of a "SERS off" [6] competitive binding type assay utilizing a plasmonic silver (AgNP) and silver coated magnetic nanoparticle (Ag@MNP) for the detection of the toxin bisphenol A (BPA). The assay scheme (Figure 1) involves a 'target' AgNP functionalized with the Raman reporter dye malachite green isothiocyanate (MGITC) and PEGylated BPA-binding DNA aptamers. These particles are designed to bind to a version of the toxin bisphenol A diglycidyl ether (BADGE), PEGylated and immobilized onto a second silver coated magnetic 'probe' nanoparticle, where binding affinities have been determined previously by Marks *et al.*[8] When mixed, target and probe nanoparticles cluster into small colloidal networks, providing a localized 'hot spot' of electric field enhancement around the RRMs at the nanoparticle interface, and therefore an increase in the number of scattering events experienced by the Raman reporter, observable as an increase in the SERS spectral intensity. This "SERS on" spectral enhancement can then be quantifiably reversed, i.e. "turned-off", by introducing free BPA to the system to compete with the aptamer BADGE interaction, causing the assemblies to release the MGITC-tagged non-magnetic target AgNPs. The assay was first characterized in on and off mode in a standard microwell plate, where assay particles are suspended in solution without magnetic manipulation. Finally Fe_2O_3 and Fe_2CoO_4 were coated in silver and tested as magnetic cores for the probe nanoparticle, where particles are housed in microfluidic chips capable of magnetically localizing the assay clusters within a PDMS channel.

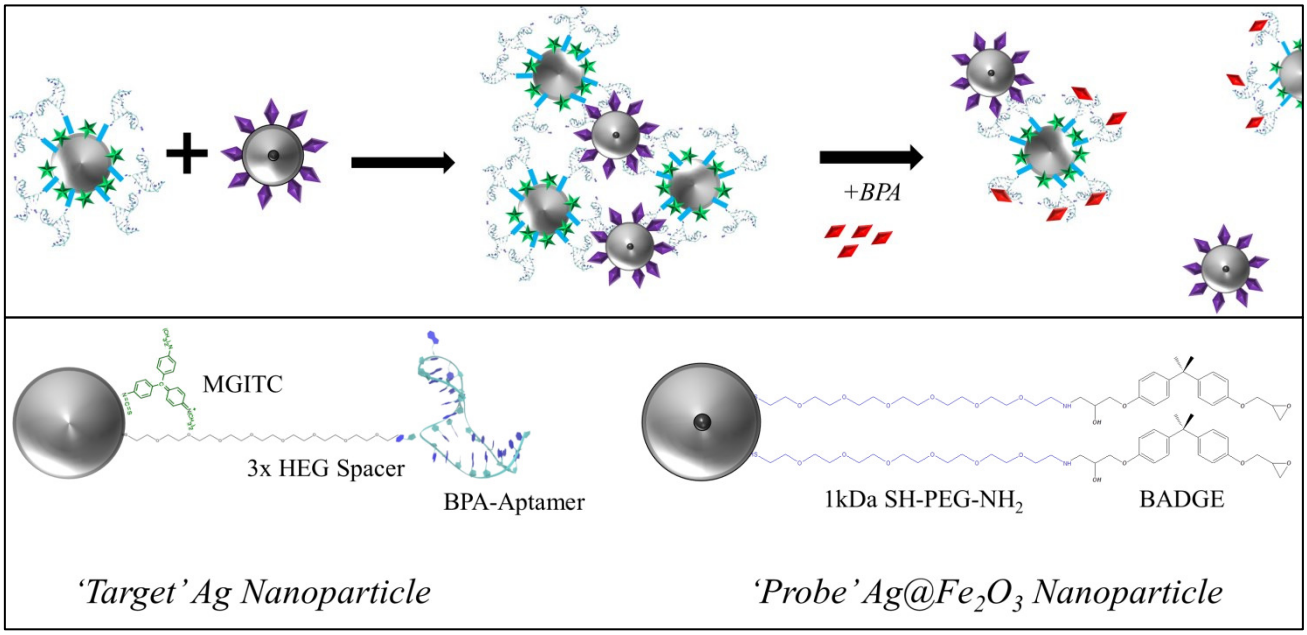

Figure 1. Top: Schematic illustration of the aptamer mediated 'turn-off' SERS competitive binding assay. Bottom: Target and probe nanoparticle conjugate components.

2. MATERIALS & METHODS

2.1 Materials and Instrumentation

The modified BPA aptamer[9]: 5'- [ThiSS][HEG]$_3$ CCG CCG TTG GTG TGG TGG GCC TAG GGC CGG CGG CGC ACA GCT GTT ATA GAC GTC TCC AGC-3' was synthesized by Integrated DNA Technologies (USA). Hetero-bifunctional PEG linker (NH_2-PEG-SH, 1kDa) was purchased from NanoCS (USA). Malachite green isothiocyanate (MGITC) reporter dye was purchased from Invitrogen (UK). All other reagents were obtained from Sigma Aldrich (USA/UK).

A Varian Cary 300Bio UV-Visible Spectrophotometer was used with a scan range of 200-800 nm for extinction measurements. The zeta potential and hydrodynamic diameters of the nanoparticles were measured on a Zetasizer Nano ZS90 (Malvern, U.K.). All microplate Raman and SERS spectra were collected using a ThermoScientific DXR Raman confocal microscope with a 900g/mm grating. SERS mapping of the microfluidic channels was done using a WITec Alpha 300R confocal Raman instrument (WITec GmbH, Ulm Germany) with a 600g/mm grating, fitted with a piezo-driven XYZ scan stage. All samples were probed using a laser wavelength of 532 nm and coupled to a thermoelectrically cooled charge-coupled device (CCD).

2.2 Colloid Synthesis

Silver colloid (AgNP) was synthesized using the method reported by Leopold and Lendl.[10] Hydroxylamine hydrochloride (1 ml, 150 mM) was added to 89 mL of NaOH (3.33 mM) under vigorous stirring. Silver nitrate ($AgNO_3$) solution (10 mL, 10 mM) was added drop-wise and stirred for 15 min at room temperature. DLS measurements revealed an average particle diameter of ~45 nm and the stock particle concentration was determined to be 225 pM according to Beer's Law using an extinction coefficient of 2.87×10^{10} M^{-1} cm^{-1} at 404 nm.[11]

Silver-coated ferrite nanoparticles ($Ag@Fe_2O_3$) were prepared using a co-precipitation method for the synthesis of the particle core and glucose reduction to coat with silver as described by Kumar *et al.*[12] and Mandal *et al.*[13] respectively. Briefly, a stock solution of maghemite (γ-Fe_2O_3) nanoparticles was prepared by adding 25 mL of an acidified iron salt solution (0.4 M Fe^{2+}, 0.8 M Fe^{3+}, 1 M HCl) drop-wise to 250 mL of 1.5 M NaOH at 50 °C under vigorous non-magnetic stirring. After 20 minutes the particles were allowed to cool, washed twice with DI water and once with 0.1M HNO_3. An additional 125 mL of HNO_3 was then added to the solution, stirred an additional 40 min at 95 °C, and resuspended in distilled water. To coat with silver, 1 mL of these stock MNPs was mixed with 4 mL of 0.35 M glucose and 1.5 mL of 60 μM $AgNO_3$. The solution was sonicated for 10 min then heated to 90 °C for 90 min. Finally, the particles were centrifuged three times to wash, and redispersed in 6 mL of 5 mM sodium citrate for a particle diameter of ~70 nm and final concentration of 425 pM.

Silver-coated cobalt ferrite nanoparticles (Ag@ Fe_2CoO_4) were prepared by first synthesizing a stock solution of the core Fe_2CoO_4 nanoparticles through co-precipitation of iron(III) chloride (0.2 M FeCl3) and cobalt(II) chloride (0.1 M CoCl2) in sodium hydroxide solution at pH ~12 (3.0 M NaOH) using a method modified from Rutirawut *et al.* [9] The cobalt ferrite salt solution was added rapidly into 3.5 M NaOH solution under vigorous stirring, then headed at 80°C for 1 h. The cobalt-iron oxide precipitated colloid was washed with deionized water in triplicate using a permanent neodymium magnet. To coat with silver, 500 μL of these stock MNPs was mixed with 4 mL of 0.35 M glucose and 1.5 mL of 60 μM $AgNO_3$. The solution was sonicated for 10 min then heated to 90 °C for 90 min. Finally, the particles were centrifuged three times to wash, and finally redispersed in 6 mL of 5 mM sodium citrate for an average particle diameter of ~35 nm and final stock concentration of 385 pM. The extinction profile of the Ag@ Fe_2CoO_4 is compared to that of $Ag@Fe_2O_3$ in Figure 2 for reference.

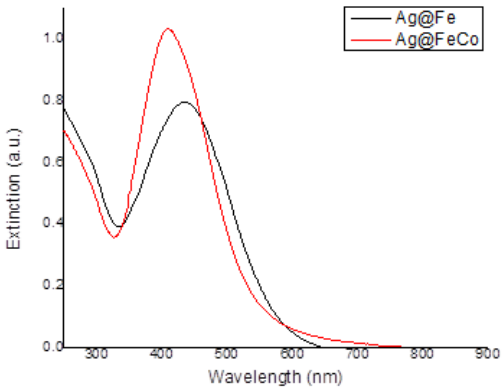

Figure 2- Comparison of the extinction profile of Fe (black) and FeCo (red) core nanoparticles after coating with silver.

2.3 Target and Probe Nanoparticle Conjugation

Aptamer/RRM AgNP Target Synthesis

Prior to immobilization, the BPA aptamers were suspended in a 60 mM phosphate buffer (PBS, pH 8.5) and treated with 15 mg of dithiothreitol (DTT) to reduce their disulfide bonds. After 1 h the aptamers were purified using a Nanoseps 10kDa desalting column to remove residual DTT, and then heated to 90 °C for 5 min to allow the aptamers to fold into their tertiary structure for optimal binding.

Target AgNPs were functionalized with aptamers using a modified method developed by Zhang et al.[14] The aptamer (7 µM) was added to 1 mL of silver colloid (145 pM) at a molar ratio of 2000:1 and left for 1 h. Three 20 µL aliquots of 250 mM citrate HCl buffer (pH 2.9) were then added 5 min apart, and the sample was left an additional 30 min before centrifuging and resuspending the aptamer functionalized particles in 1 mL of 0.3 M PBS (pH 7.4). The Raman reporter molecule, malachite green isothiocyanate (MGITC, 10 µM in methanol), was then added at a 500:1 ratio and left to react with the aptamer AgNPs under sonication for 1 h, which were then centrifuged and stored in a 0.1 M PBS buffer (pH 7.4).

Analyte Ag@MNP Probe Synthesis

To synthesize 1 mL of BADGE functionalized magnetic nanoprobes, the SH-PEG-NH_2 linker was added drop-wise to an excess of the analyte BADGE in 0.3 M PBS buffer (pH 8.5) and left overnight to allow conjugation between their terminal amine and epoxide groups respectfully. The conjugates were then treated with aminoethanol to open unbound epoxide rings and prevent non-specific binding of BADGE to the RRM's amine groups. These SH-PEG-BADGE conjugates were then added at a 5000:1 ratio to 1 mL of silver coated magnetic nanoparticles (stocks diluted to 225 pM). After 1 h three 20 µL aliquots of 250 mM citrate HCl buffer were again added 5 min apart, and the sample was left an additional 30 min before centrifuging and resuspending the BADGE functionalized particles in 1 mL of 0.1 M PBS (pH 7.4).

2.4 Fabrication of Ni-patterned magnetic microchannel

The magnetic channel-based SERS device is composed of a nickel micromagnet array, microfluidic channel and permanent magnet. The Ni-micromagnet array is constructed on a glass slide through lithography as depicted by the fabrication process chart in Table 1. Glass slides are spin-coated with LOR 3A and S-1813 lift-off resists at 750 nm and 1.3 µm, respectively. After exposure and development, the pattern of the Ni-micromagnet array is visibly transferred onto the glass slides. Next, 100 nm-thick chromium and 50 nm-thick copper are continuously deposited on the pattern as the adhesion layer and then 200nm-thick nickel is deposited as the 3rd and final magnetic response layer. After deposition, the entire glass slide is placed into the chemical stripper of LOR 3A at 80°C in order to remove the photoresist layer. A 100um-thick microfluidic channel is constructed using a silicon wafer mold via soft lithography. Uncured-PDMS solution (10:1) is poured over the silicon wafer mold and cured at 65°C for 2 hours. Lastly, the PDMS microfluidic channel is bound to the glass slide patterned with a micromagnet array by plasma etching treatment. The nickel pads within the channel are not magnetically activated until the final 3D printed neodymium magnet holder is placed around the channel.

2.5 SERS analysis of assay in a microwell

A solution containing equal volumes of 225 pM of each of the target and probe nanoparticles was monitored with SERS for 5 h to allow complete binding between the immobilized BPA aptamers and BADGE. The assembled nanoparticle clusters were collected using a neodymium magnet held at the side of the glass vial, the supernatant containing any unbound AgNPs was removed, and the assembled nanoparticles were resuspended in 0.1 M PBS (pH 7.4). SERS measurements were collected with the target AgNP concentration held constant at 75 pM in a total volume of 30 µL of 0.1 M PBS (pH 7.4) with an integration time of 10 s (1s exposure). For competitive binding analysis 10 µL of the assembled target/probe NPs was mixed with 10 µL of 1 µM BPA in and 10uL of BPA (1 pM to 1uM) in 0.1 M PBS (pH 7.4) and SERS measurements were taken every 30 s.

2.6 SERS analysis of pre-bound assay particles in a magnetic microfluidic

A solution containing equal volumes of 225 pM of each of the target and probe nanoparticles in a total volume of 300uL was allowed to flow through the Ni-patterned magnetic channels at a flow rate of 10 µL/min, and sub-sequentially left overnight. The SERS profile of the localized assembled nanoparticle clusters was mapped using WiTec analysis software. Specifically SERS measurements were collected with an integration time of 1 s (1s total exposure) and a step size of 10 µm for a total 3D mapping area of 150 x 150 x 250 µm.

3. RESULTS AND DISCUSSION

The target nanoparticles were functionalized as described and yielded average zeta potential measurement of -39.7 mV. Probe particles of the Fe and FeCo varietals were found to be -32.1 mV and -22.7 mV respectively, indicating that the PEG spacer and the HEG modified aptamer provide adequate particle stabilization in the 0.1 M PBS binding buffer, but that the Co doped particles were slightly less stable. The intrinsic SERS signal of a stock solution of the assembled nanoparticles as it and its response to the presence of competing BPA was then analyzed in a microwell using the Ag@Fe particles. As shown by Figure 3, the SERS signal of the reporter molecule MGITC increases by 25 orders of magnitude as it experiences an enhancement in the electric field intensity of its immediate environment due to the aptamer-binding induced particle aggregation, reaching a steady-state equilibrium after ~ 2 h.

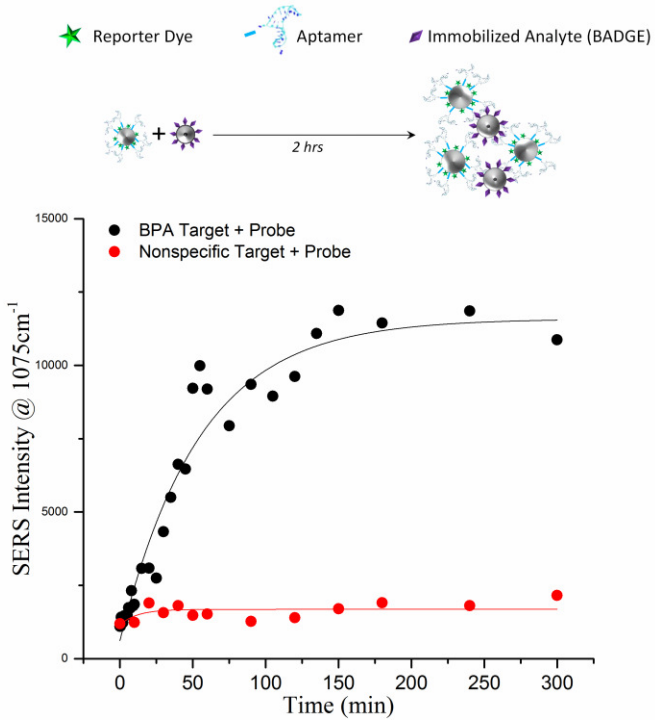

Figure 3. SERS spectral intensity of the 1075 cm-1 Raman mode from target nanoparticle, conjugated to either a BPA specific or nonspecific aptamer sequence, monitored for 5 h after exposure to the probe nanoparticles at a 1:1 molar ratio.

When free BPA is added to the enhanced conjugate solution, it is observed that the SERS intensity of MGITC's 1175 cm^{-1} peak (aromatic C–H bending vibrational mode[3]) decreases to a steady state over the course of ~3 min as competitive binding occurs (Figure 4). This implies that the BPA aptamer immobilized on the target AgNPs loosens or releases from the BADGE on the MNPs in order to more favorably bind to free BPA, causing a decrease in the solution SERS signal due to MGITC being displaced further from the nanoparticle surface interface as hypothesized.

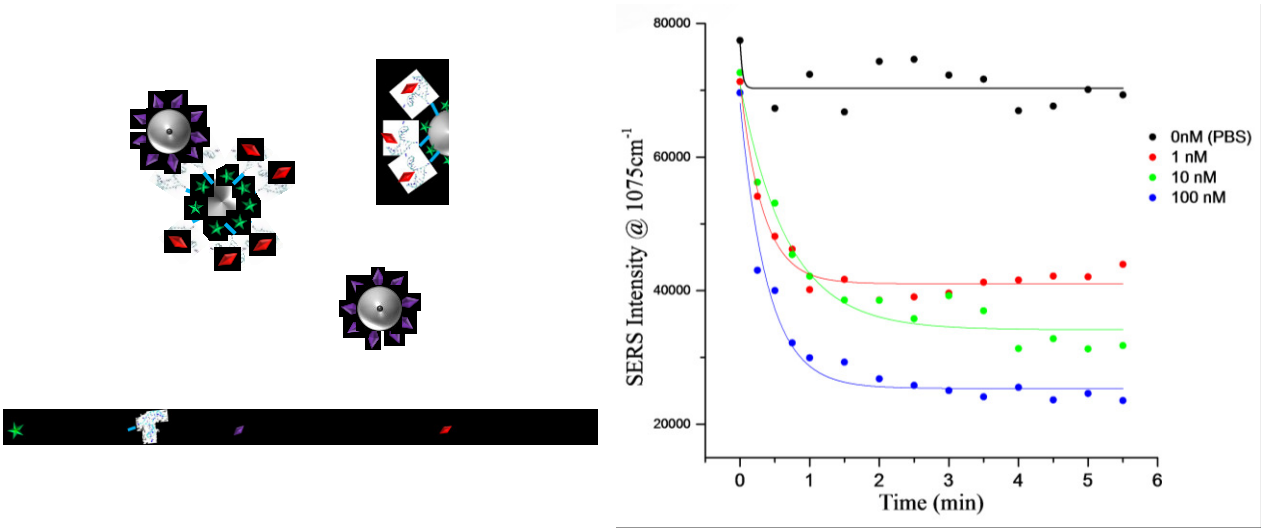

Figure 4. Schematic and SERS spectra of the target and probe nanoparticle assembly NPs and the assay response after exposure to 0-100 nM of the competing analyte BPA in free solution.

In order to create a more repeatable Raman analysis platform for assays relying on magnetic nanoparticles we use a magnetically activated Ni-patterned optofluidic chip to house the sensing chemistry. Specifically, we compared the magnetic collection rate and monitored the SERS enhancement of silver coated Fe2O3 and Fe2CoO4 nanoparticles for two different Ni-pattern designs shown in Figure 5: straight (top image) and spotted (bottom image). Using 3D Raman mapping, we found that the straight channel provided a more uniform nanoparticle organization with a coefficient of variation of 10-20% compared to ~35% variation across the ten Ni-pads within the channel walls (Fig 5, Raman intensity maps). On a positive note the spotted channels demonstrated a larger SERS enhancement, and thus a lower limit of detection, and in investigating the error across each individual pad it was discovered that the error could eb reduced through normalization to the pads' maximum intensity, for an improved % error in the 10-20% range. Additionally, the Ag@ Fe2O3 particles were, on average, more uniform in size and more stable than Ag@ Fe2CoO4, however the addition of cobalt did significantly improve the collection time of particles from several hours down to ~20 minutes within the magnetic chips and thus may still worth investigating further.

4. CONCLUSION

We have described a "turn-off" SERS assay platform methodology for the detection of small toxins utilizing aptamer-mediated assembly of SERS active colloidal nanoparticles. The colloidal SERS assay is formed through the mixing of probe and target nanoparticles and reaches an equilibrium after ~2 hours. We were then able to monitor a three minute competitive binding event initiated by the presence of as little as 10µL of 1 nM bisphenol A in free solution. It was determined that the probe nanoparticles have specific preference for the BPA aptamer target particles, and that the particles competitively bind with free BPA as well, implying that no nonspecific binding occurred. This approach has the potential to be translated to any aptamer/antigen pair and provides the added benefit of magnetic manipulation of the nanoparticle sensing network to remove any unbound nanoparticles that could potentially interfere with the Raman readout.

Our magnetic microfluidic chip is designed for simple integration with other lab-on-chip devices, such as those for sample pre-processing for biological fluids. We found that the Ag@ Fe_2O_3 particles were, on average, larger yet more uniform in size and more stable than Ag@ Fe_2CoO_4, while the addition of cobalt significantly improved the collection time of particles within the magnetic chips. Using 3D Raman mapping, we found that the straight channel design with the Ag@ Fe_2O_3 particles intrinsically provided the most uniform nanoparticle organization, while the spotted channel design with Ag@

Fe_2CoO_4 particles demonstrated a larger SERS enhancement, and thus a lower overall limit of detection. It was found that the pad to pad variability could be reduced through normalization to the maximum intensity within a pad, and thus the spotted design used in conjunction with the Fe_2O_3 magnetic core varietal of the probe nanoparticle was the most successful iteration of the experiment. Due to the intrinsic properties of colloidal plasmonic nanoparticles this platform technology could potentially be used with multiple optical detection schemes such as fluorescence, absorbance, and Raman spectroscopies and can be tuned to virtually any biomarker-sensing ligand pair- welcoming continuous innovation after these initial embodiments.

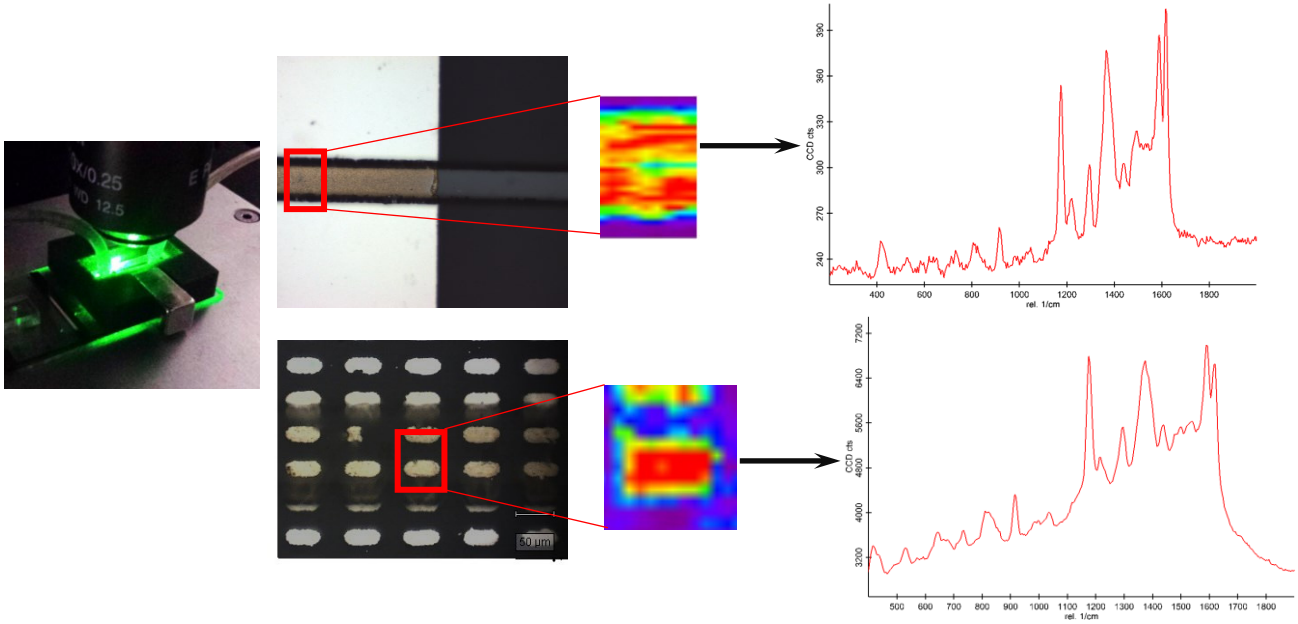

Figure 5. From left to right: 1) image of the experiment setup for Raman mapping of the assay nanoparticle clusters within the mangetic microfluidic, 2) brightfield images of the Ni-patterned detection regions through a 10x objective for the straight (top) and spotted (bottom) designs, 3) Raman maps of Fe-core (top) and FeCo-core (bottom) type assay particles in the 'SERS on' state, and 4) full SERS spectra of the red regions of the Raman maps.

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