Supporting Information

Quantitative Multiplexed Simulated-Cell Identification by SERS in Microfluidic Devices

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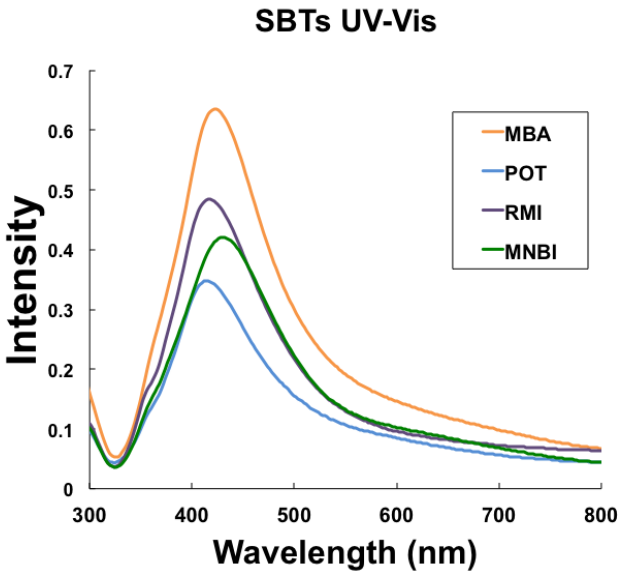

Figure S1. The UV-Vis spectra of the synthesized SBTs. The intensity of the 408 nm peak is related to the concentration of silver nanoparticles in each solution. This band was used to normalize the concentration of SBTs to ensure comparable SERS intensities.

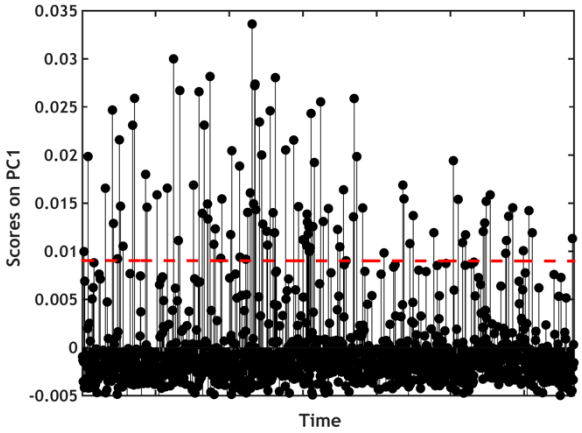

Figure S2. The filtering PCA (fPCA) results for one run. Each point represents a measurement in the time-point SERS interrogation. The fPCA algorithm performs an initial PCA on the data and returns a 1-PC model in which the PC1 score is directly associated with the contribution of SERS peaks in the spectrum as apposed to the relatively constant contribution of PDMS Raman bands. The threshold for filtering the PDMS-only spectra was set at the 95% confidence limit of the PC1 scores data and was adjusted manually based on the signal-to-noise ratio of the spectra.

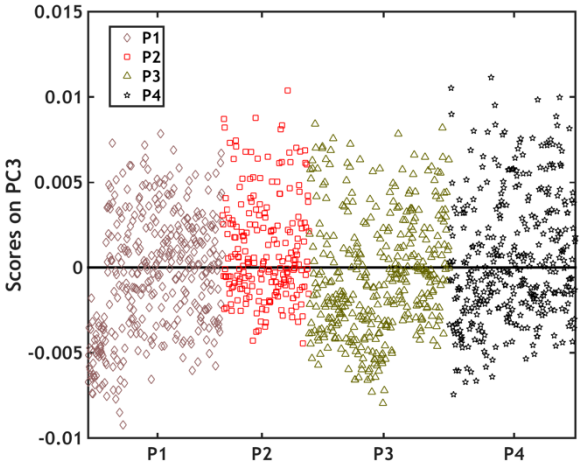

Figure S3. PC3 scores calculated by the PCA model based on the spectra from all four populations. PC3 does not show a significant variation between populations.

Table S1. Summary of normalized CLS scores vs. nominal concentrations of SBTs

	MBA-SBT CLS Score	MBA-SBT nominal conc.	POT-SBT CLS Score	POT-SBT nominal conc.	RMI-SBT CLS Score	RMI-SBT nominal conc.	MNBI-SBT CLS Score	MNBI-SBT nominal conc.
Population 1	6±0.1%	0%	3±0.1%	0%	75±0.7%	75%	15±0.3%	25%
Population 2	47±0.8%	50%	21±0.5%	25%	9±0.2%	0%	22±0.6%	25%
Population 3	10±0.1%	0%	38±0.4%	50%	35±0.6%	25%	18±0.3%	25%
Population 4	19±0.6%	25%	18±0.4%	25%	40±0.6%	25%	22±0.4%	25%

Device Fabrication

Microfluidic-based, flow-focusing devices were designed and constructed on the principles described by Stiles et al[1], such that the length of channels determines the flow rates and the width of the focused stream. The devices were made of polydimethylsiloxane (PDMS, Sylgard 184) using an SU-8 mold fabricated by soft lithography. PDMS was cast on the mold and cured for 60 minutes at 80 ˚C, peeled off and access holes cut using a biopsy punch. The resulting devices were ozone treated to enhance bonding, then sandwiched between two glass microscope slides. Holes were previously drilled in the backside glass slide and pipette tips were inserted to serve as reservoirs and vacuum tubing interfaces. The flow was actuated by a diaphragm vacuum pump (Gast Manufacturing Corporation) connected to the outlet of the device.

SBT synthesis

The procedure for the SBTs synthesis has been described in detail previously.[2] The silver colloid was synthesized according to the Lee and Meisel protocol:[3] 500 ml of deionized water (DI, resistivity 18 MΩ) with 1 mM silver nitrate (Sigma, 99.999%) were brought to a boil. Then, 10 ml of 1% trisodium citrate dihydrate (Sigma) were added. The mixture was kept at boiling temperature for about 60 min until the color turned dark green/gray. Aliquots of the colloid were taken and centrifuged at 1600×g to remove the smallest particles. The yellow supernatant was

discarded and the pellet was resuspended in deionized MilliQ water (DI) and diluted until the absorbance of the band at 408 nm was 0.4 at 0.1 mm path length. The resulting colloid was named Ag04. SBTs were then prepared by adding to every 100 µL of Ag04 3 µL phosphate buffer (250 mM, pH 7.5), 6 µL hexamethylenediamine (Sigma, 98%, 0.4 mg/ml in DI, pH 4.0), waiting for 2 minutes, then adding 6 µL of 1% polyvinylpyrrolidone 40 kDa (Sigma) in DI and 100 µL DI, and, finally waiting for 5 minutes then adding 2 µL of 6.5 mg/mL BSA-SPDP in 0.1x PBS (Bovine serum albumin –BSA- purchased from Sigma was conjugated to N-succinimidyl 3-(2-pyridyldithio)-propionate, (SPDP, Pierce #21857) so that the modified protein interacts with the Ag surface strongly through the SPDP molecule with the pyridine group appearing in the SERS spectrum prior to Raman reporter being added). The particles resulting from this controlled aggregation stage were called AgPHPB and were then used to prepare SBTs for use with beads by adding the appropriate Raman reporter (MBA, POT, RMI, and MNBI at a final concentration of 100 µM, 200 µM, 20 µM, and 100 µM, respectively) and waiting for 60 minutes. MBA, POT, and MNBI were purchased from Sigma and RMI from Santa Cruz Biotechnology. SVA (succinimidyl valerate)-PEG5kDa-biotin (LysanBio) was then added to a final concentration of 1mg/ml of colloid, and allowed to incubate for 4 hours at room temperature. After adding 0.005% final concentration of Tween-20 (T20 - polyoxyethylene (20) sorbitan monolaurate solution 10% in H_2O, Sigma), the SBTs were washed twice by centrifugation (15 minutes @ 800×g), the supernatant was discarded (to remove most of the non-SERS-bright silver monomers still in the solution) and the pellet resuspended in 1/20th the initial volume in 0.1x PBS/0.1 % BSA/ 0.005% T20 after the second centrifugation step.

Bead/SBT incubation

6 µm streptavidin coated polystyrene beads (Polysciences, PA, USA) were diluted in buffer (0.1xPBS + 0.1%BSA + 0.005% T20) at a final concentration of $5x10^6$ beads/mL. SBT cocktails

were prepared by mixing them at the desired ratios and added to the diluted beads at 10 μL per 100 μL of sample. The mixture was incubated overnight on a tube rotator at room temperature and washed twice by centrifugation at 100×g for 6 minutes to remove unbound SBTs. Biotin was added to the samples at a final concentration of 0.3 μM and incubated for 15 minutes in between the two wash steps to block any unreacted streptavidin sites on the beads. The bead suspension was supplemented with 18% (by vol) OptiPrep (Sigma) as a density matching agent, before being injected into devices for SERS measurements.

SERS Measurements

The SERS measurements were performed on a LabRAM Aramis spectrometer (Horiba Jobin-Yvon, Kyoto, Japan), using a 633 nm excitation laser. A 10x objective was used with 11 mW power at the interrogation spot. SERS signatures of SBT-labeled beads flowing through the microfluidic channel were acquired every 100-150 ms with a 20 ms nominal detector exposure time. However the effective exposure time is the time that a flowing particle resides within the laser beam, and it depends on the flow rates, which were set such that each bead resides within the focused laser beam for 5-8 ms as it flows across the device. The slit and hole were set to 250 μm and 600 μm, respectively and a 600 gr/cm grating was used.

Data Analysis

All chemometric analysis was performed using the PLS Toolbox (Eigenvector Research, Inc., Wenatchee, WA) for MATLAB (Mathworks, Inc., Natick MA). Filtering PCA (fPCA) was used as a preprocessing step on all the acquired time point data to remove PDMS-only spectra. It is basically a model with 1 PC (fPC) that contains all the peaks associated with the set of spectra being analyzed. Spectra with high score in fPC contain SERS peaks from SBTs whereas the spectra with zero, or close to zero fPC score mainly show peaks from PDMS and were filtered out. The threshold for filtering was set based on the fPC scores 95% confidence limit and was

adjusted manually based to the signal-to-noise ratio of the spectra. PCA was performed on the filtered data set to categorize the populations' spectra in the 280-1800 cm^{-1} range using the single value decomposition (SVD) algorithm. Spectral deconvolution was carried out using a CLS model that was constructed using the SERS spectra of each one of the pure SBTs (i.e. not in a cocktail) in the range of 800-1800 cm^{-1}, where no major bands from PDMS are present. Both PCA and CLS analysis were preceded by a preprocessing step including baseline subtraction, normalization based on area under the spectrum, and for PCA mean-centering was then performed. The percent composition of each SBT in the four populations calculated by CLS is expressed as the mean value of the normalized computed scores ± standard error of the mean. Nonparametric analysis of variance (Kruskal–Wallis test) was performed on the CLS scores of the individual SBTs across all populations to test the statistical different between groups using MATLAB. Sample numbers for all the statistical analyses: population1 N=386, population2 N=249, population3 N=403, and population4 N=363.

1 T. Stiles, R. Fallon, T. Vestad, J. Oakey, D. W. M. Marr, J. Squier and R. Jimenez, *Microfluid. Nanofluid.*, 2005, **1**, 280–283.
2 A. Pallaoro, G. B. Braun and M. Moskovits, *Proc. Natl. Acad. Sci. U S A*, 2011, **108**, 16559–16564.
3 P. C. Lee and D. Meisel, *J. Phys. Chem.*, 1982, **86**, 3391–3395.