# ACOUSTOFLUIDIC FOCUSING OF PS@Ag: TOWARDS CONTINUOUSLY REFRESHING SERS SUBSTRATE

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**Abstract**

Acoustic forces enable contactless and selective manipulation of particles within microchannels, allowing precise control over their distribution and flow dynamics. This work presents the development of a glass microfluidic chip with a piezoelectric element, designed for the continuous handling of functionalized polystyrene microparticles using acoustofluidic principles. The core innovation lies in the design and implementation of a system that facilitates controlled mixing of the sample with these microparticles, followed by their spatial concentration in a dedicated detection zone. As part of the system development, substantial effort was devoted to optimizing the synthesis and surface functionalization of polystyrene microparticles (PS) with silver nanostructures (PS@Ag), intended for future use as SERS-active carriers. Although surface-enhanced Raman spectroscopy (SERS) detection is not yet implemented, this study establishes the groundwork by demonstrating the feasibility of continuous particle focusing and transport within the microfluidic platform. The presented device represents a crucial step toward the realization of an automated, flow-through analytical system, with the potential for future integration with separation techniques and real-time, SERS-based chemical analysis.

**Keywords:** Acoustofluidics, manipulation, nanoparticles, Raman spectroscopy, substrate

## 1. INTRODUCTION

Raman spectroscopy is a powerful analytical technique for specific compound identification through vibrational fingerprints, obtained by monitoring inelastically scattered photons. While versatile, its inherent low sensitivity due to a small scattering cross-section limits its wider use in separation methods.

This sensitivity limitation is largely overcome by surface-enhanced Raman spectroscopy (SERS), which greatly amplifies the Raman signal. [1] On the SERS substrates (i.e., nanostructures from silver/gold), localized surface plasmons are created after the interaction with incident laser light. This enhances the incident laser's electric field resulting in the enhancement of analyte signal and allows highly sensitive detection, even at single-molecule levels, especially at *hot spots* with exceptionally strong electromagnetic fields.

SERS substrates are typically either dispersed nanoparticles (NPs) or nanostructured solid surfaces. Dispersed nanoparticles, commonly synthesized by chemical reduction methods, offer flexibility in experimental design and are often used by inducing controlled aggregation to form *hot spots* for signal enhancement. [2–4] Their potential can be further extended via chemical modification of the surface or their chemical composition. [5] A significant challenge for dispersed nanoparticles is their stability, especially in high ionic strength solutions, where rapid aggregation can lead to signal instability and issues like channel clogging. [6] In contrast, nanostructured solid substrates involve firmly anchored metallic nanostructures, which generally provide better stability and repeatability of the SERS signal. [7] However, achieving reproducible morphology during their often complex fabrication is crucial, and rapid, complete desorption of analytes from immobilized substrates remains a practical difficulty, leading to memory effects in online applications. [8]

In this work, we immobilized silver nanoparticles on the polystyrene microparticles (PS@Ag) to combine advantages of dispersed systems with embedded SERS substrates. We developed a simple glass microfluidic Y-junction addressing three important issues: (1) the device allows continuous supply of a sample and PS@Ag with potential to be fully automated, (2) the detection in the flow allows continuous renewal of the SERS substrate minimizing the possible memory effect, and finally (3) the device operates with acoustofluidic forces focusing the PS@Ag into the center of the channel, which increases concentration of silver in the detection point.

Indeed, acoustofluidics is a technique within microfluidics that uses ultrasonic waves to manipulate particles in microchannels. [9,10] When a sound wave encounters a reflected wave, it can create a resonant acoustic standing wave (ASW). This standing wave generates phenomena within the fluid. Variations in fluid velocity create a drag force known as acoustic streaming. Simultaneously, a pressure gradient between the nodes and anti-nodes of the wave produces an acoustic radiation force. This force causes particles to migrate. How a particle is affected depends on the differences in physical properties between the particles and the liquid. The versatility of this method comes from its reliance on mechanical properties, making it nearly independent of chemical factors like pH or charge.

## 2. EXPERIMENTAL

### 2.1. Synthesis of composite structures (PS@Ag, PS@Au)

The composite structures were formed by a simple co-incubation of polystyrene (PS) microspheres with pre-synthesized metallic nanoparticles. The PS microspheres (5 μm diameter, 10% solid content) were purchased from Supelco.

Silver nanoparticles were prepared via the chemical reduction of silver nitrate (Lach-Ner) by hydroxylamine hydrochloride (Sigma-Aldrich) under alkaline conditions. Specifically, 0.53 mL of a 2 mM silver nitrate solution was rapidly injected into a vigorously stirred 3.74 mL solution containing 3.2 mM NaOH (Penta) and 0.8 mM hydroxylamine. The mixing was terminated after 15 minutes. Gold nanoparticles were reduced from gold(III) chloride trihydrate (Darmstadt) by sodium citrate tribasic dihydrate (Darmstadt). A 50 mL solution of 0.29 mM gold(III) chloride was heated to boiling and maintained for 5 minutes. Subsequently, 1 mL of a 38.8 mM sodium citrate solution was added dropwise. The mixture was boiled for an additional 6 minutes.

Prior to the formation of the composite structures, the PS microspheres were thoroughly washed with deionized water to remove stabilizers. The AgNP and AuNP dispersions were then concentrated by centrifugation at 10,000 RCF for 6 minutes using a microcentrifuge (MiniSpin Plus, Eppendorf). A suitable volume of the supernatant was removed to achieve a final approximate metal concentration of 135 μg/mL. Finally, 995 μL of the concentrated AgNP or AuNP dispersion was mixed with 5 μL of the purified PS microsphere suspension to spontaneously form PS@Ag or PS@Au, respectively.

### 2.2 Characterization of dispersions

The morphology of the composite microspheres was characterized using scanning electron microscopy (SEM; Mira3, Tescan). Images were acquired at an acceleration voltage of either 5 kV or 10 kV with a beam intensity of 12 eV. The final images were generated by combining signals from the in-beam (90%) and secondary electron (10%) detectors. Additional characterization was performed using a UV-Vis spectrophotometer (Shimadzu-1800), with deionized water serving as the blank reference.

### 2.3 Microfluidic device design and fabrication

The microfluidic chip was fabricated from borosilicate glass slides (Schott). The device was constructed using standard photolithography and wet etching techniques. The channels were etched in a HF-based solution to a

final depth of 40 µm. The chip consisted of two patterned glass slides (**Figure 1A**), which were thermally bonded to seal the microfluidic channels (**Figure 1B**). Prior to bonding, inlet and outlet ports were drilled into the top slide. Liquids were supplied to the chip through fused-silica capillaries (ID 75 µm, length 65 cm) connected with specialized fittings (LabSmith). Fluid flow was driven by applying a pressure of 0.1 atm. To generate the acoustic field, a PZT piezoceramic plate (Pz26, Ferroperm Piezoceramics) was affixed to the top surface of the assembled chip, approximately 1-2 mm from the main channel (**Figure 1C**). The transducer was operated at a frequency of 6.820 MHz to generate the acoustic waves.

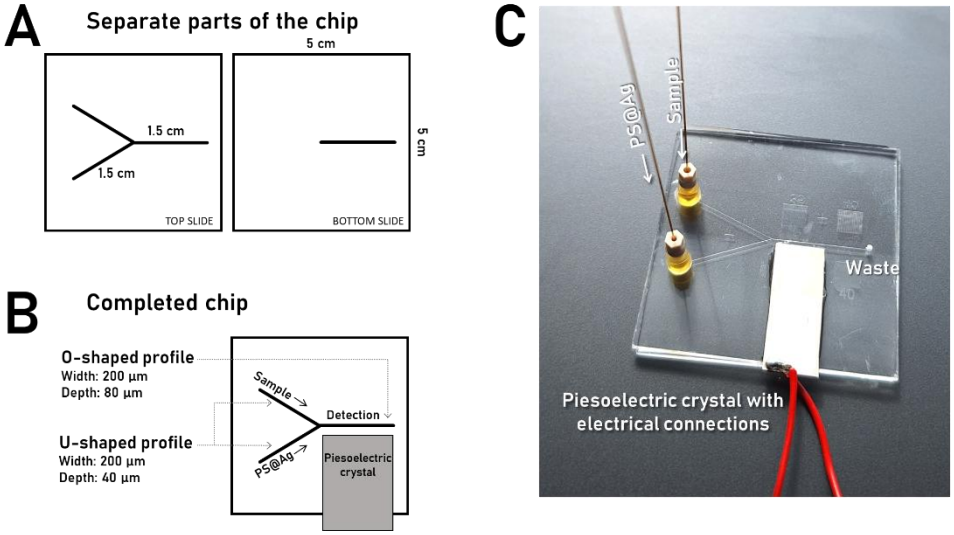


**Figure 1** A – Design on separate glass slides used for the completion of the chip. B – Scheme of the fabricated chip. C – Picture of the complete microfluidic chip.

## 3. RESULTS AND DISCUSSION

The primary motivation for this study was the development of a feasible synthetic pathway for a SERS substrate that combines the advantages of dispersed nanoparticles with those of embedded substrates. We hypothesized that immobilizing plasmonic nanoparticles onto the surface of PS microspheres would form highly organized structures. These structures could be easily flushed from microfluidic channels, thereby minimizing the memory effect. Furthermore, the high-density arrangement of nanoparticles would create numerous *hot spots* without the need for a chemical aggregating agent. An additional objective was the acoustophoretic concentration of these PS@Ag composites at the detection point to ensure a sufficient quantity of roughened surface for robust SERS detection.

We discovered that the formation of PS@Ag is surprisingly facilitated by a spontaneous affinity between the AgNPs and the commercial PS microspheres. A simple co-incubation of the two components resulted in excellent surface coverage on the 5-µm microspheres. To investigate the kinetics of this interaction, aliquots were taken from the reaction mixture at three time points: (1) immediately after mixing, (2) after 15 minutes, and (3) after 60 minutes of incubation. Comparative analysis of SEM images of the resulting PS@Ag composites revealed that the PS-AgNPs interaction is rapid, occurring almost instantaneously (**Figure 2A**). The surface coverage was found to be practically identical across all three samples, indicating its independence from the co-incubation time. For comparison, **Figure 2B** shows the bare surface of a PS microsphere from a control sample without AgNPs. It should be noted that the non-conductive nature of the PS sphere led to slight shape deformation under the electron beam.

The spontaneous attachment of AgNPs to the PS surface is particularly noteworthy and unexpected, as both particle types typically exhibit a strong negative zeta potential. Consequently, electrostatic attraction is unlikely

to be the primary interaction mechanism. We hypothesize that the attachment of AgNPs to the PS surface may be mediated by residual stabilizing agents, such as polymers, which are often anchored to the surface of PS microspheres during their synthesis. [11] These stabilizing structures commonly possess functional groups capable of engaging in numerous, albeit weak, van der Waals interactions, which collectively could be strong enough to firmly attach the silver nanostructures to the microsphere surface.

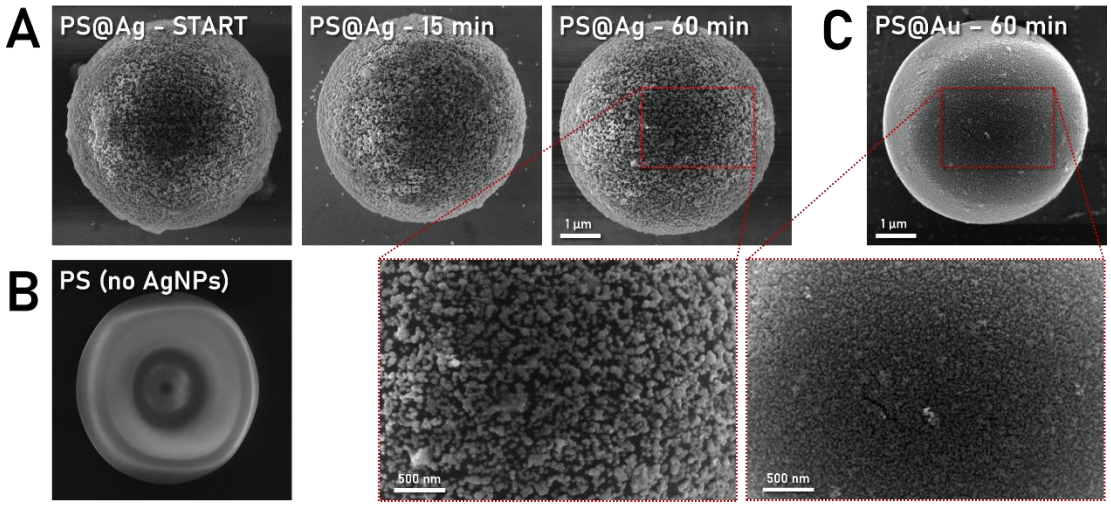


**Figure 2** SEM figures. A – The effect of co-incubation time on the surface coverage of PS microspheres by AgNPs. B – Blank sample; PS microsphere without AgNPs. C – PS@Au.

The tendency of the PS microspheres to spontaneously adsorb plasmonic nanoparticles was also tested with gold nanoparticles (AuNPs). The procedure for obtaining PS@Au composites was identical to that used for the PS@Ag structures, with a co-incubation time of 30 minutes. The resulting PS@Au surface, shown in **Figure 2C**, demonstrates an excellent affinity between the AuNPs and the PS microspheres. Due to the considerably smaller dimensions of the AuNPs used, the nanostructures populated the exposed PS surface more effectively, leading to improved surface coverage compared to the AgNPs. Although gold nanoparticles offer advantages for biological applications due to their superior biocompatibility, the inherently stronger surface enhancement capability silver makes PS@Ag structures more suitable for routine analytical measurements, and therefore we employ PS@Ag in further work.

As the plasmonic properties of the PS@Ag composites are crucial for their application in SERS, the dispersion was characterized by UV-Vis absorption spectroscopy (**Figure 3A**). The absorption spectrum of the PS@Ag composite dispersion was compared to that of the constituent silver colloid and a PS microsphere dispersion at equivalent concentrations. The pure silver colloid exhibited a characteristic absorption spectrum with a maximum at 410 nm, which is typical for localized surface plasmon resonance of individual AgNPs. In contrast, the PS dispersion showed a baseline increase in absorbance across the visible range, likely due to light scattering by the microspheres. Notably, the spectrum of the PS@Ag dispersion revealed a significant decrease in the absorption maximum at 410 nm and a broad increase in absorption across the rest of the monitored spectrum. This spectral change indicates the coupling of plasmons between closely packed AgNPs on the microsphere surface [12,13], which we consider to be independent proof of successful PS@Ag formation.

Finally, we investigated the suitability of the microfluidic device for the continuous preparation and analysis of the PS@Ag SERS substrate. The device was designed as a simple Y-junction with two inlet channels and a main detection channel featuring acoustophoretic manipulation capabilities. To facilitate particle focusing, the detection channel was composed of two overlapping channels forming a profile with ellipse cross-section. A key objective was to optimize the position of the SERS detection point downstream from the Y-junction to allow

for both (1) sufficient diffusion of the analyte into the stream of PS@Ag composites and (2) adequate time for the acoustophoretic migration of the composites to the channel centerline. This optimization was performed using auxiliary fluorescence measurements with a dye solution (**Figure 3B**) and fluorescently-labeled PS microspheres. Based on these experiments, a position 8 mm downstream from the junction was selected as the minimal distance that satisfied both conditions.

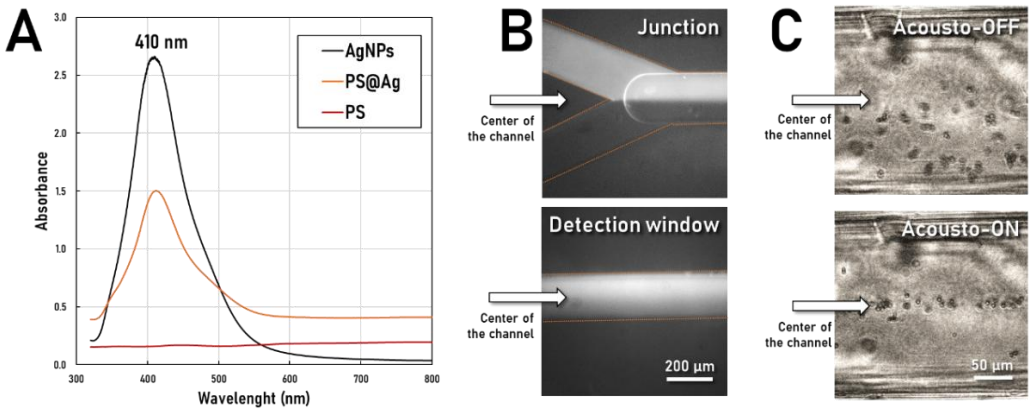


**Figure 3** A – UV-Vis absorption spectra of selected dispersions. B – Microscopic figures of fluorescence flow of Rhodamine B at two various positions on the chip. The channels of the wall are visualized by orange lines. C – Microscopic figures of channel in the distance of 8 mm from the junction at ON/OFF acoustophoretic conditions.

The acoustofluidic manipulation of the PS@Ag composites was demonstrated by applying an acoustic wave at an optimized frequency (**Figure 3C**). In the absence of the acoustic field (Acousto-OFF), the particles remained confined to their initial streamline, filling approximately half of the channel. This behavior is characteristic of the laminar flow regime for particles with a low diffusion coefficient. However, upon activation of the acoustic field (Acousto-ON), the resulting acoustophoretic forces directed the migration of the microspheres to the channel's center, where they formed a tightly focused stream.

## 4. CONCLUSION

This study successfully developed a novel acoustofluidic system for the continuous manipulation and focusing of PS@Ag microspheres within a glass microfluidic chip. A core innovation was to create SERS-active carriers, combining advantages of dispersed and embedded substrates to mitigate memory effects through continuous substrate renewal. The synthesis process revealed a surprisingly spontaneous and rapid affinity between AgNPs/AuNPs and commercial PS microspheres, a finding independently confirmed by both SEM imaging and UV-Vis absorption spectroscopy. Within the microfluidic platform, acoustophoretic forces were effectively demonstrated to concentrate the PS@Ag composites into a tightly focused stream at the channel's centerline. This foundational work establishes a crucial step towards realizing an automated, flow-through analytical system with significant potential for future real-time, SERS-based chemical analysis.

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***The original experimental data are available: https://doi.org/10.57680/asep.0638297***

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