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Supplementary Information: Submicron- and Nanoplastic Detection at Low Micro- to Nanogram Concentrations Using Gold Nanostar-Based Surface-Enhanced Raman Scattering (SERS) Substrates

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Table S1: A summary of relevant measurement parameters for all Raman and SERS spectra presented in the study. All measurements were obtained using a 785 nm excitation laser line. Spectra for submicron- and nanoplastics were collected using a 50x objective, while microplastics were studied with a 20x objective.

Sample	Substrate	Grating (gr/mm)	Laser Power (mW)	Number of Accumulations	Integration Time (s)
20 µg/mL of 161 nm PS	AuStars	1200	2	150	0.5
10 µg/mL of 161 nm PS	AuStars	1200	6	250	0.5
5 µg/mL of 161 nm PS	AuStars	1200	2	150	0.5
2.5 µg/mL of 161 nm PS	AuStars	1200	5	200	0.5
1.25 µg/mL of 161 nm PS	AuStars	1200	12	500	0.5
625 ng/mL of 161 nm PS	AuStars	1200	5	500	0.5
20 µg/mL of 33 nm PS	AuStars	1200	6	250	0.5
10 µg/mL of 33 nm PS	AuStars	1200	4	250	0.5
5 µg/mL of 33 nm PS	AuStars	1200	5	200	0.5
2.5 µg/mL of 33 nm PS	AuStars	1200	2	150	0.5
1.25 µg/mL of 33 nm PS	AuStars	1200	4	750	0.5
20 µg/mL of 36 nm PET	AuStars	1200	2	150	0.5
10 µg/mL of 36 nm PET	AuStars	1200	2.5	50	0.5
5 µg/mL of 36 nm PET	AuStars	1200	1	150	0.5
126 nm PE Stock	AuStars	1200	1.5	350	0.5

121 nm PP Stock	AuStars	1200	1	400	0.5
PS MP	Glass	300	10	250	0.5
PET MP	Glass	300	10	250	0.5
PE MP	Glass	300	10	250	0.5
PP MP	Glass	300	6	300	0.5
161 nm PS Stock	Glass	300	12	250	0.5
33 nm PS Stock	Glass	300	12	250	0.5
36 nm PET Stock	Glass	300	40	200	1
20 µg/mL of 161 nm PS	Glass	1200	2	150	0.5
10 µg/mL of 161 nm PS	Glass	1200	6	150	0.5
5 µg/mL of 161 nm PS	Glass	1200	10	200	1
2.5 µg/mL of 161 nm PS	Glass	1200	5	200	0.5
1.25 µg/mL of 161 nm PS	Glass	1200	12	500	0.5
625 ng/mL of 161 nm PS	Glass	1200	5	200	0.5
20 µg/mL of 33 nm PS	Glass	1200	6	250	0.5
10 µg/mL of 33 nm PS	Glass	1200	4	250	0.5
5 µg/mL of 33 nm PS	Glass	1200	10	200	1
2.5 µg/mL of 33 nm PS	Glass	1200	5	200	0.5

1.25 µg/mL of 33 nm PS	Glass	1200	10	200	0.5
20 µg/mL of 36 nm PET	AuSpheres	1200	2.5	150	0.5
126 nm PE Stock	AuSpheres	1200	1	300	0.5
121 nm PP Stock	AuSpheres	1200	2.5	300	0.5
20 µg/mL of 36 nm PET	Glass	1200	2.5	150	0.5
10 µg/mL of 36 nm PET	Glass	1200	4	150	0.5
5 µg/mL of 36 nm PET	Glass	1200	1	150	0.5
Control	Glass	300	40	100	1
Control	AuStars	300	1	250	0.5
Control	AuSpheres	300	2.5	150	0.5

Table S2: A summary of Raman peaks used to identify each plastic type and their assignments. In the mode assignments, *v = stretching,* v_s *= symmetric stretching,* v_{as} *= asymmetric stretching, and δ = bending.*

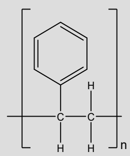
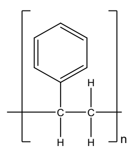
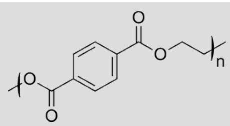
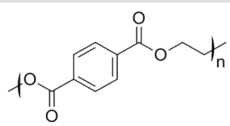
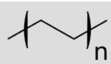
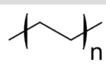
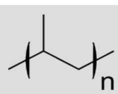
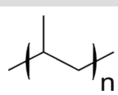
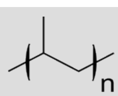
	Polymer Chemical Structure	**Raman Shift (cm^{-1})**	**Vibrational Mode**	**Assignment**
PS		1002	*v12*(C-C-C)	Ring breathing mode (1, 2)
PS		1032	*v18a*(C-H)	CH in-plane deformation (1, 2)
PET		1615-1620	*δ8a*(C-C-C)	Aromatic bending vibrations (1, 3)
PET		1730	*v*(C=O)	Carbonyl stretching mode (1, 4)
PE		2850	$v_s(CH_2)$	CH_2 symmetric stretching vibrations (4)
PE		2883	$v_{as}(CH_2)$	CH_2 asymmetric stretching vibrations (4)
PP		2840	$v_s(CH_2)$	CH_2 symmetric stretching vibrations (4, 5)
PP		2883	$v_{as}(CH_2)$	CH_2 asymmetric stretching vibrations (4, 5)
PP		2952	$v_{as}(CH_3)$	CH_3 asymmetric stretching vibrations (4, 5)

Table S3: A detailed summary of all SERS literature mentioned within the discussion section "Comparison of SERS Substrates for Plastic Particle Detection".

Study	SERS Substrate / Excitation Wavelength	Plastic Particles Analyzed	LODs Reported (µg/mL)	Notes on Sample Matrix
Present Study	LbL Assembled AuStars on glass / 785 nm	PS (161 nm, 33 nm)	0.625, 1.25	All experiments done with dried samples, initial dispersions in Milli-Q water
		PET (36 nm)	5	
		PE (126 nm)	Not detectable	
		PP (121 nm)	Not detectable	
	LbL Assembled AuSpheres on glass / 785 nm	PET (36 nm)	20	
		PE (126 nm)	Not detectable	
		PP (121 nm)	Not detectable	
Caldwell, J.	LbL Assembled	PS (161 nm, 33 nm)	10, 20	All experiments done with dried

et al. 2021 (1)	AuSpheres on glass / 785 nm	PET (62 nm)	15	samples, initial dispersions in Milli-Q water
Kihara *et al.* 2022 (6)	20 nm AuSpheres on filter paper / 785 nm	PS (200 nm, 20 nm)	5 - 10	All experiments done with samples dried via filtering, with initial dispersions in ultrapure water.
Yang *et al.* 2022 (7)	Ag nanowires on a filter / 633 nm	PS (1 µm, 500 nm, 300 nm, 100 nm, 50 nm) PMMA (500 nm)	0.0001 0.001	The LODs reported were for plastics in pure water with KI, dried after filtering through the substrate and measured in air. Other measurements were done in sea water and river water.
Xu, G. *et al.* 2020 (8)	Klarite / 785 nm	PS (5 µm, 2 µm, 1 µm, 500 nm, 360 nm) PET (~450 nm)	~26.25 (tested for all) Not reported	All experiments done with dried samples PS and PMMA initially in Milli-Q water

		PMMA (5 µm, 2 µm, 500 nm, 360 nm)	~26.25 (tested for all)	PET was an atmospheric sample
Xu, D. *et al.* 2022 (9)	AuSphere-doped filter paper / 532 nm	PET (20 µm, 15 µm, 10 µm)	100	LODs reported for dried PET samples initially dispersed in Milli-Q with surfactant Other tests done with tap and pond waters
Yin *et al.* 2021 (10)	AuSphere-soaked sponge / 785 nm	PS (150 - 80 µm) PET (150 - 80 µm) PE (150 - 80 µm) PP (150 - 80 µm)	1 Not reported Not reported Not reported	Particles were initially dispersed in ultrapure water, then dried to measure. LODs are reported for these samples. Other tests done with sea water, rainwater, river water, snow water, and tap water

Mikac *et al.* 2023 (11)	Aggregated AuSpheres or Au nanorods / 785 nm	PS (350 nm) PE (4 – 1 µm)	6.5 200	PS samples in water were mixed with Au nanorods and an "aggregating agent", then dried to measure PE particles were prepared the same way, but with the addition of surfactant in the initial plastic dispersion
Lv *et al.* 2020 (12)	Aggregated AgSpheres / 785 nm	PS (500 nm, 100 nm) PE (10 µm) PP (10 µm)	40 (lowest tested for both) 5000 (lowest tested) 5000 (lowest tested)	Measurements were conducted with PS in dispersed state in ultrapure water, with AgSpheres aggregated around plastic particles using NaCl to obtain the LODs reported here. PE and PP samples additionally contained surfactant. Other measurements were done in

			sea water

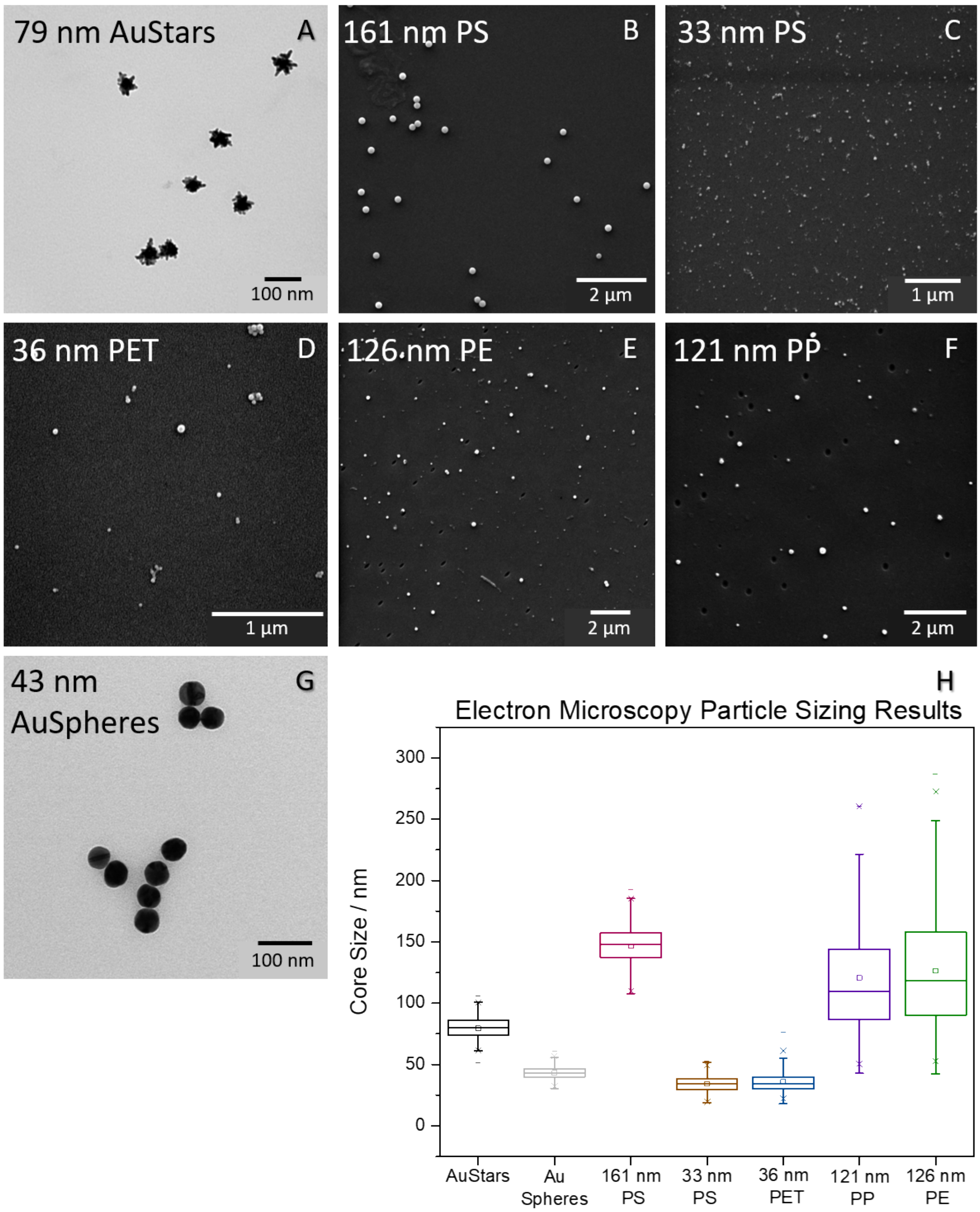


Figure S1: Representative electron microscopy images for each particle type presented in this study. A) A TEM image of AuStars. B) An SEM image of 161 nm PS submicronplastics. C) An SEM image of 33 nm PS nanoplastics. D) An SEM image of 36 nm PET nanoplastics. E) An SEM image of 126 nm PE submicronplastics. F) An SEM image of 121 nm PP submicronplastics. G) A TEM image of AuSpheres. H) A box plot of sizing data for each particle type. Boxes represent first to third quartile data, with the mean *value indicated by the smaller, open square. Median values are indicated by a central line within the larger boxes, and whiskers indicate the minimum or maximum values.*

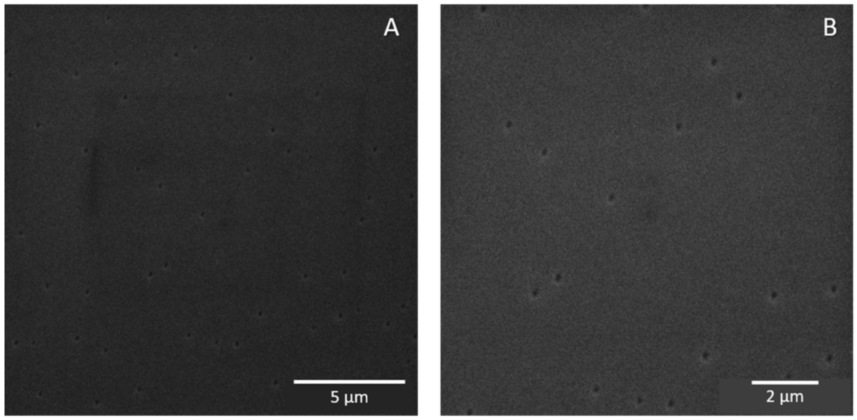


Figure S2: Controls from SEM imaging of blanks which show no particulate matter contamination at two different magnifications.

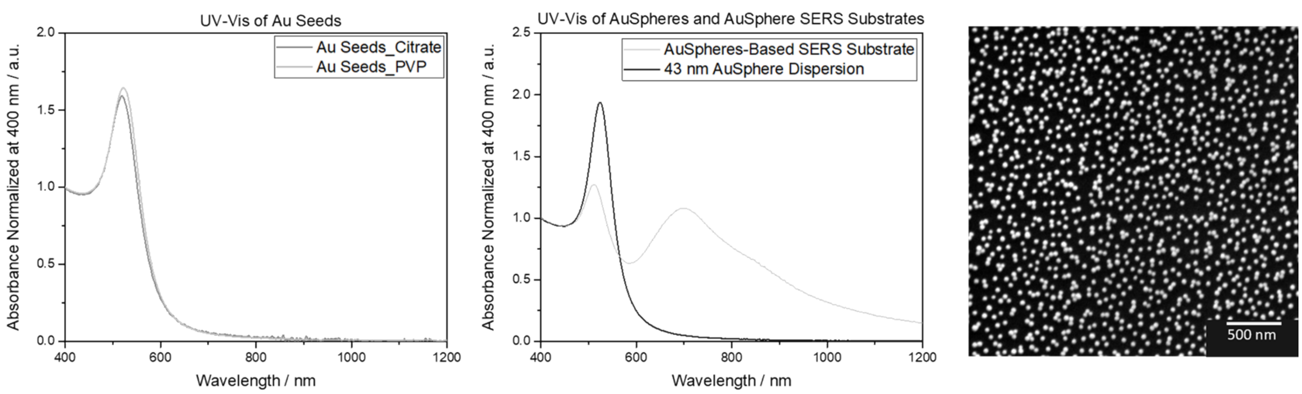


Figure S3: An overview of the plasmonic properties of the gold seeds and 43 nm AuSpheres. 43 nm AuSpheres are shown both in dispersion and assembled to create SERS substrates. Left: A UV-Vis spectrum of gold seeds for AuSphere synthesis (_Citrate) and gold seeds for AuStar synthesis (_PVP). Center: A UV-Vis spectrum of the AuSpheres in Milli-Q water compared with a UV-Vis spectrum of a SERS substrate. Right: A representative SEM image of an AuSpheres-based SERS substrate.

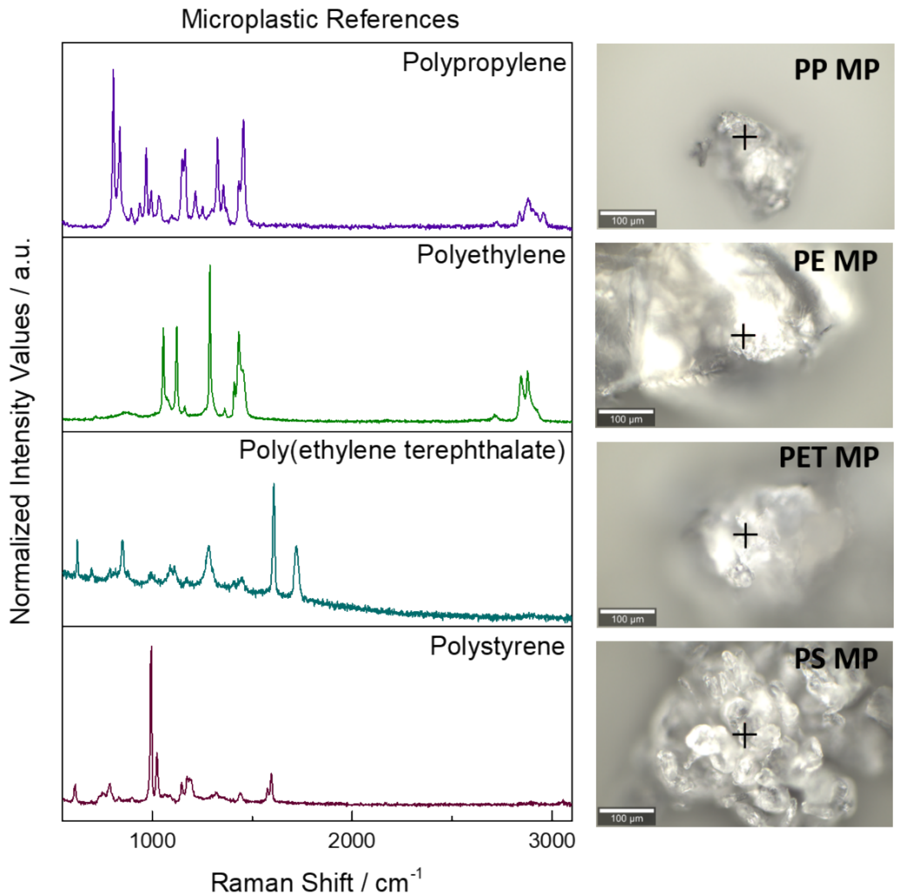


Figure S4: Reference microplastic particles (MPs) for each type of plastic used in the study. Left: Stacked Raman spectra for PS, PET, PE, and PP MPs. Right: Brightfield images with black crosses indicating the regions of interest that the Raman measurements were taken at. Scale bar is 100 µm for all samples. Full measurement parameters for each sample can be viewed in Table S1.

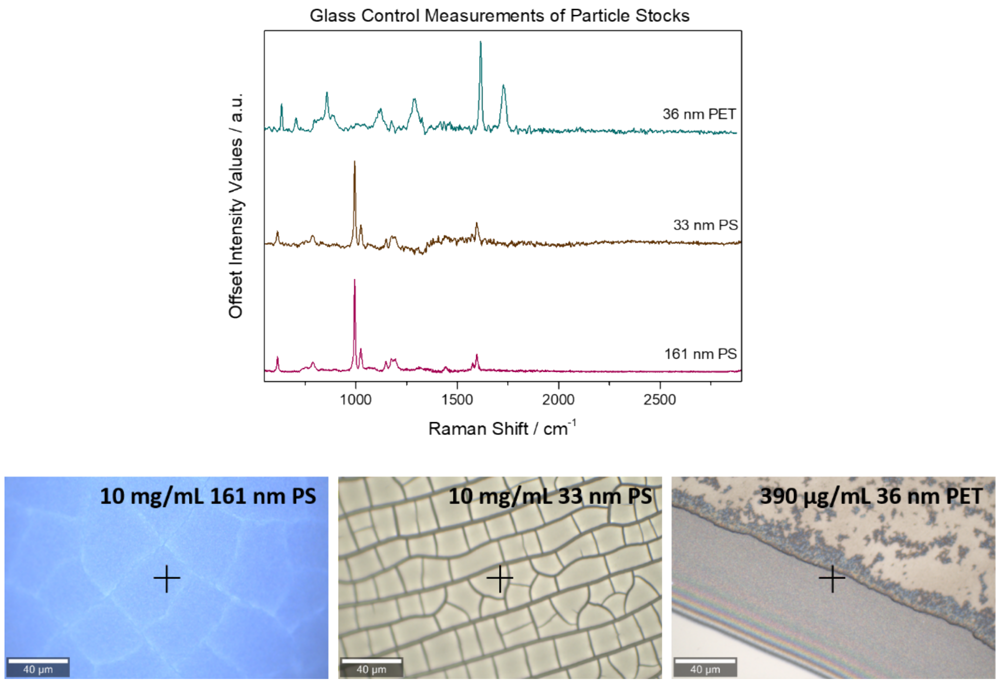


Figure S5: Control measurements of the initial submicron- and nanoplastic stocks dried on glass for all particles that could be detected using this method. Top: Raman spectra for both 161 nm and 33 nm PS particles as well as the 36 nm PET particles. Bottom: Brightfield images showing the region of interest (with black crosses) that the Raman measurements were obtained from. Scale bar is 40 µm for all samples. Full measurement parameters for each sample can be viewed in Table S1.

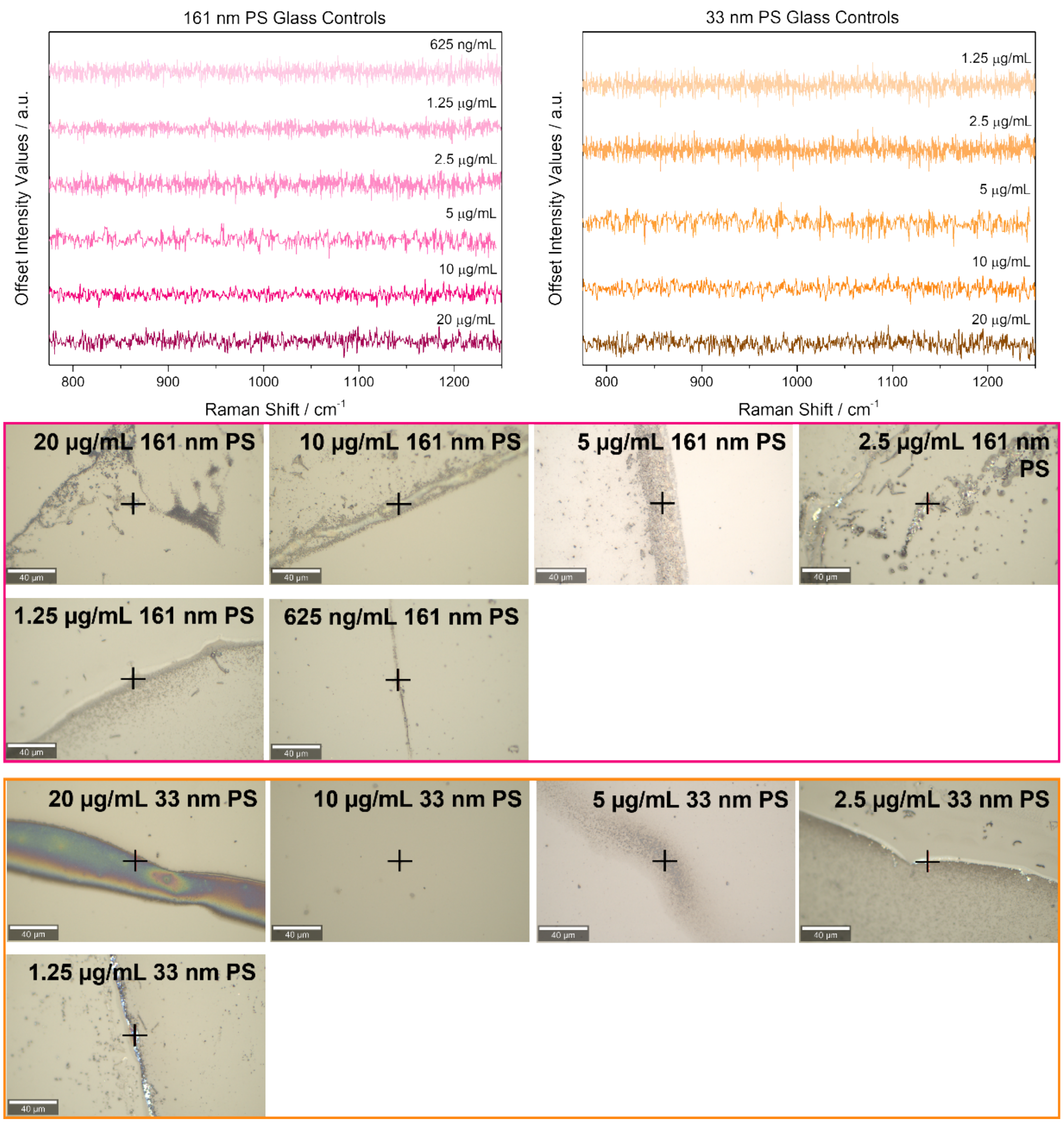


Figure S6: Control data for PS particles on plain glass. Upper left: Regular Raman spectra for all concentrations it was possible to detect the 161 nm PS submicronplastics at using AuStars-based substrates. Upper right: Regular Raman spectra for all concentrations it was possible to detect the 33 nm PS nanoplastics at using AuStars-based substrates. Bottom: Brightfield images of the regions of interest that the SERS spectra were obtained from. Exact measurement positions are indicated with black crosses. Scale bar is 40 µm for all samples. Full measurement parameters for each sample can be viewed in Table S1.

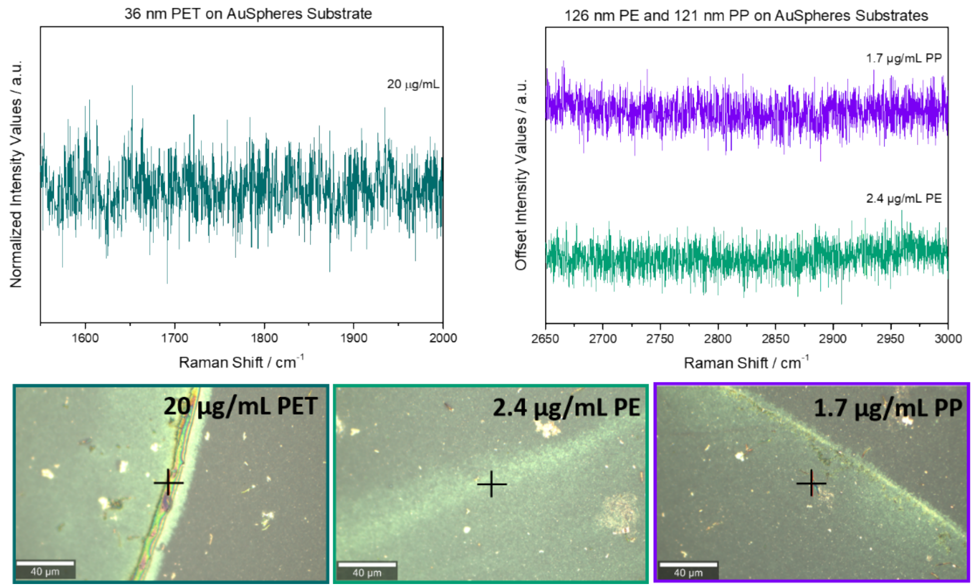


Figure S7: SERS data for PET, PE, and PP particles on AuSpheres substrates. Upper left: SERS spectra for the highest concentration of 36 nm PET nanoplastics detected on AuStars-based substrates. Upper right: SERS spectra for the PE and PP submicronplastics which could not be observed on either substrate type. Bottom: Brightfield images of the regions of interest that the SERS spectra were obtained from. Exact measurement positions are indicated with black crosses. Scale bar is 40 µm for all samples. Full measurement parameters for each sample can be viewed in Table S1.

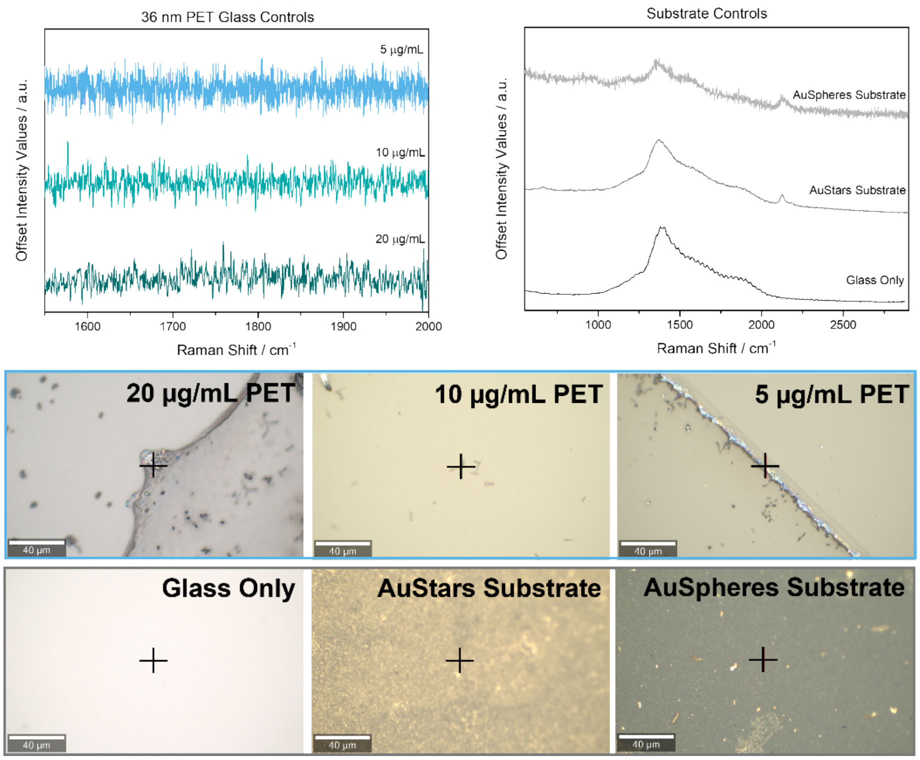


Figure S8: Control data for PET particles on plain glass and for each type of substrate without a plastic sample. Upper left: Regular Raman spectra for all concentrations it was possible to detect the 36 nm PET nanoplastics at using AuStars-based substrates. Upper right: Spectra for plain glass, AuStars-based SERS substrates without plastic particles, and AuSpheres-based SERS substrates without plastic particles. Bottom: Brightfield images of the regions of interest that the SERS spectra were obtained from. Exact measurement positions are indicated with black crosses. Scale bar is 40 µm for all samples. Full measurement parameters for each sample can be viewed in Table S1.

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