Towards Raman automation for microplastics: developing strategies for particle adhesion and filter subsampling.

AUTHORS

Clara Thaysen[1*], Keenan Munno[1], Ludovic Hermabessiere[1,2], Chelsea Rochman[1]

[1]Department of Ecology and Evolutionary Biology, University of Toronto, Canada

[2]Department of Civil and Mineral Engineering, University of Toronto, Canada

*corresponding author, clara.thaysen@gmail.com

TABLE OF CONTENTS

Section 1: Skin Tac filtration test times; Raman spectra from Skin Tac interference tests

Section 1a: *Skin Tac filtration test times*

Table S1: Filtration times for 100 mL of RO water through a 47 mm diameter, 10 µm pore size polycarbonate (PC) filter (Millipore Sigma, Oakville, ON, Canada) both with and without Skin Tac (Torbot Group Inc., Cranston, RI, USA) applied.

	Filtration time (s)	
Replicate	**WITHOUT SKIN TAC**	**WITH SKIN TAC**
1	5	9
2	5	10
3	5	13
AVERAGE TIME (±standard deviation)	**5 (0)**	**10.6 (2.1)**

Table S2: Filtration times for 500 mL of RO water through a 47 mm diameter, 10 µm pore size PC filter (Millipore Sigma) both with and without Skin Tac (Torbot Group Inc.) applied.

	Filtration time (s)	
Replicate	**WITHOUT SKIN TAC**	**WITH SKIN TAC**
1	15	40
2	13	54
3	11	68
AVERAGE TIME (±standard deviation)	**13 (2.0)**	**54 (14)**

Section 1a: ***Raman spectra from Skin Tac interference tests***

Table S3. The Raman identification and HQI (score out of 100) for materials used in the proposed filtering procedure (47 mm diameter, 10 µm pore size PC filters, Skin Tac wipes) as well as reference materials (PET fibres, multi-polymer fragment stock solution) mounted using the proposed filtering technique (n=3). The polymer identification was assigned based on the Raman ID and corresponding HQI. The laser used to acquire each spectrum is provided.

Material	**Laser (nm)**	**Raman ID**	**HQI (/100)**	**Polymer ID**
Clean PC Filter	532	Poly(Bisphenol A carbonate) + 12-DOXYL-stearic acid	89.55	PC
	532	Poly(Carbonate bisphenol A-based)	98.89	PC
	532	Poly(Carbonate bisphenol A-based)	98.71	PC
Skin Tac + PC Filter	532	Poly(Bisphenol A carbonate)	97.88	PC
	785	p-(Carbonate)	96.81	PC
	785	Poly(Carbonate bisphenol A-based)	97.07	PC
Skin Tac Wipe (Fibre)	532	Anatase + Cotton 3a. Yellow fibre	97.26	Cellulosic
	532	Cotton 3a. Yellow fibre	91.92	Cellulosic
	532	Cotton 3a. Yellow fibre	95.70	Cellulosic
PET Fibre (Mounted on Skin Tac + PC Filter)	785	Polyester 7. Red fabric	89.78	PET
	785	Polyester 7. Red fabric	91.05	PET
	785	Polyester 7. Red fabric	89.94	PET
Multi-Polymer Fragment (Mounted on Skin Tac + PC Filter)	532	HDPE 2287	98.27	PE
	785	Polystyrene 6. Clear glassware	98.12	PS
	785	Polyester 12. Red fibre	87.59	PET

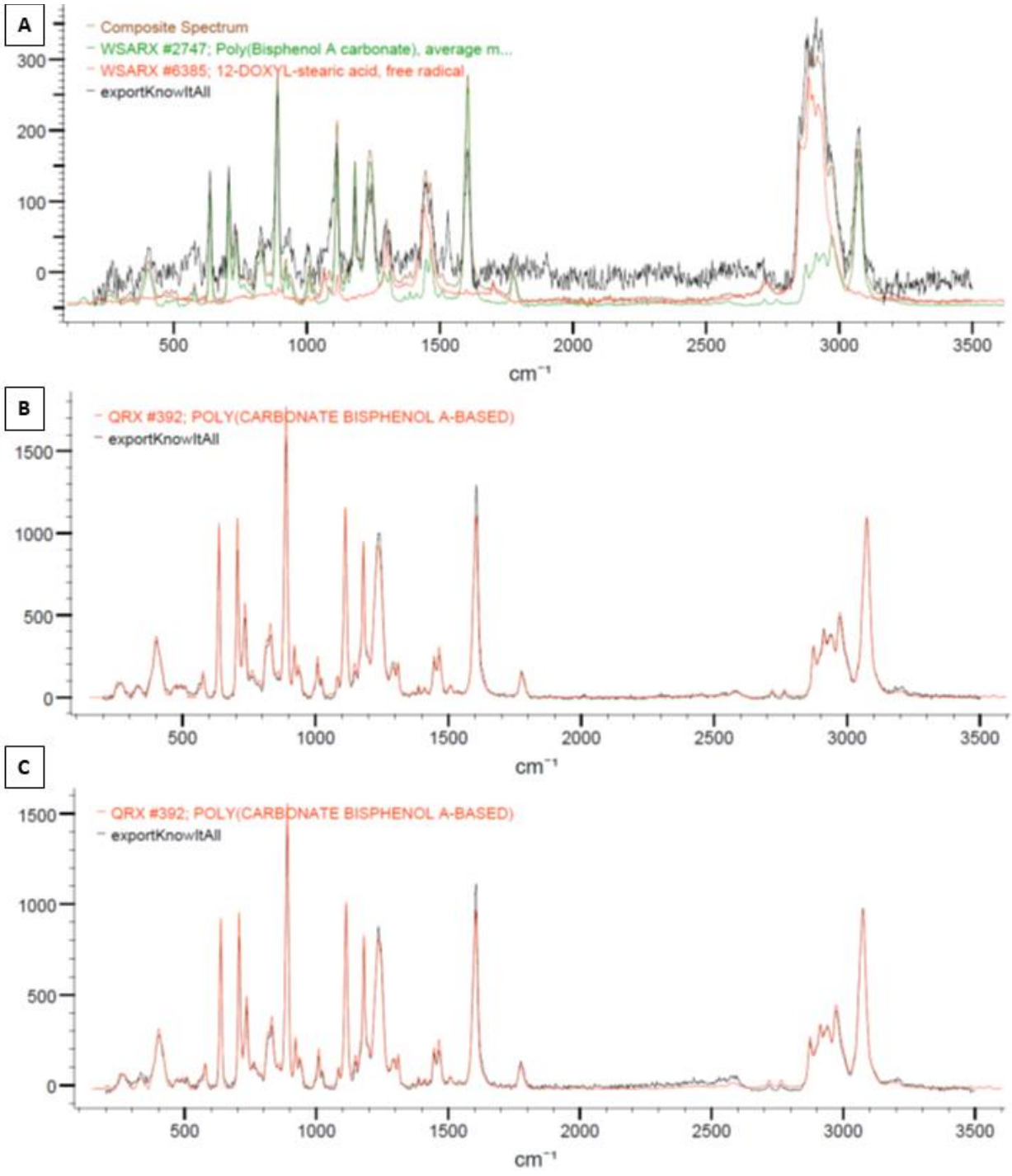


Figure S1. An unused 10 µm PC filter was analyzed using Raman spectroscopy to confirm it is polymeric composition (*N*=3; a, b, c). The filter material was polycarbonate.

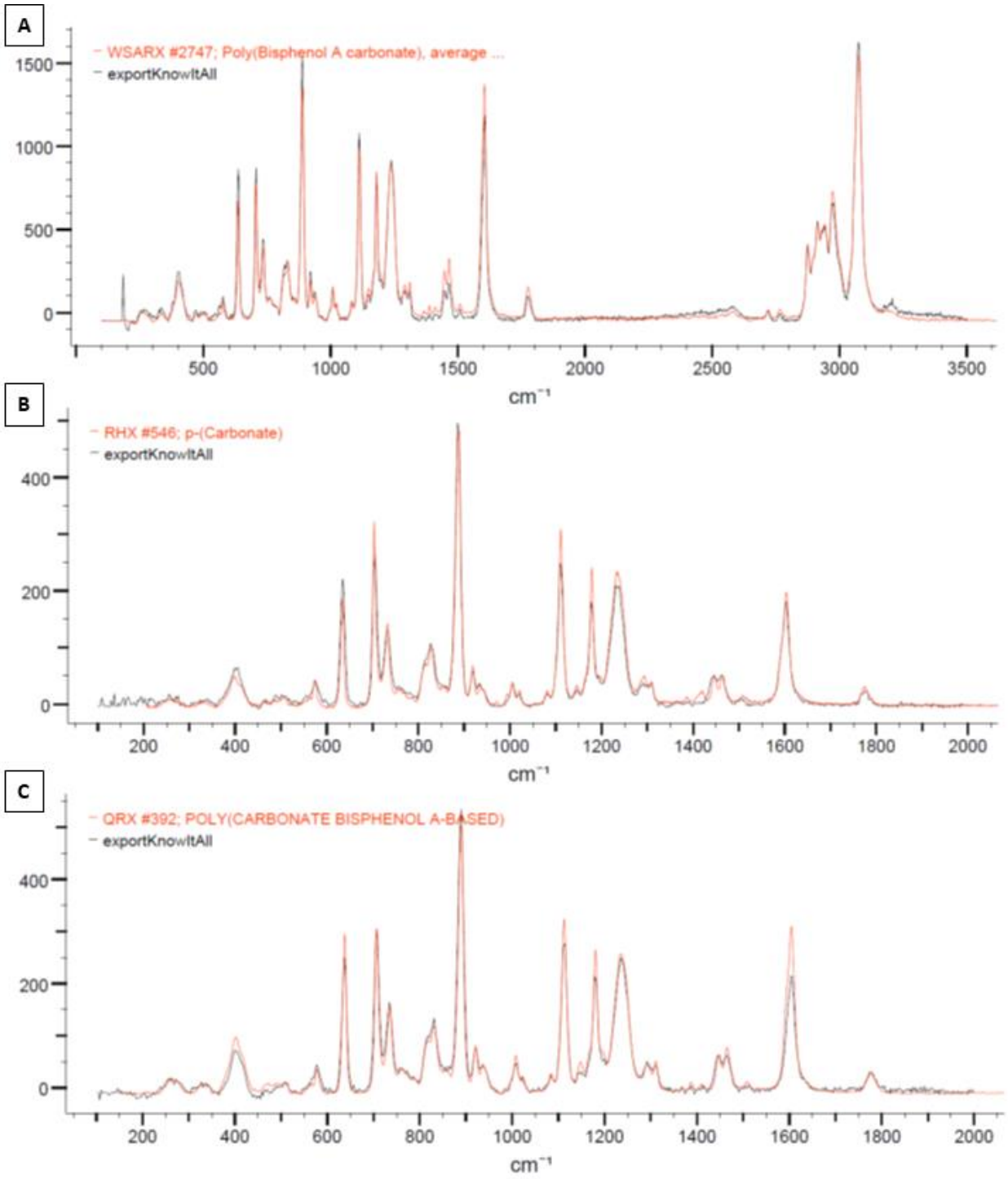


Figure S2. A 10 µm PC filter was wiped with Skin Tac and the filter surface was analyzed using Raman spectroscopy to determine whether the Skin Tac produces a strong Raman signal (*N*=3; a, b, c). The resulting spectra did not appear to contain peaks for rosin, the adhesive in Skin Tac wipes.

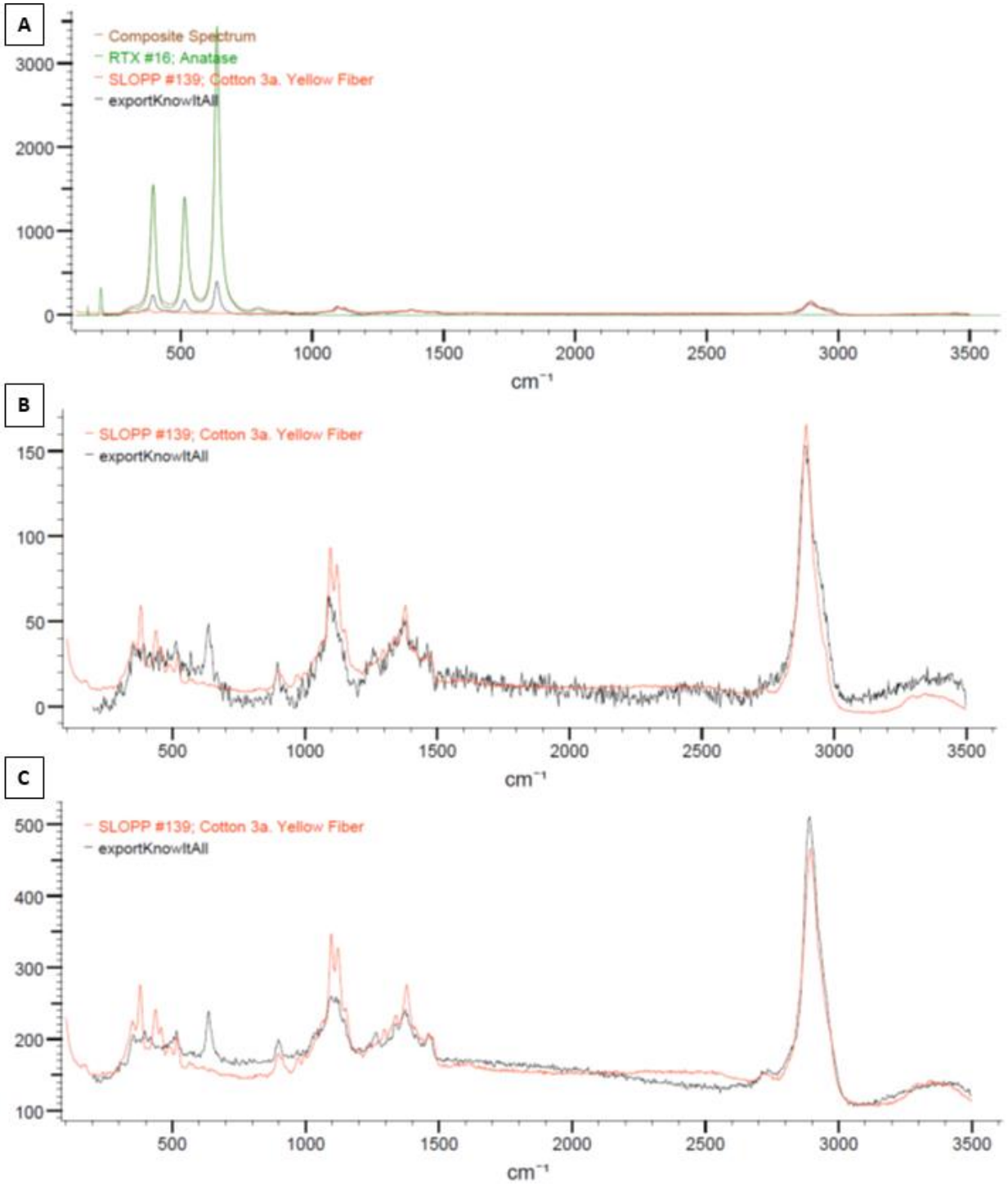


Figure S3. Fibres from Skin Tac wipes were assessed using Raman spectroscopy to identify their polymeric composition (*N*=3; a, b, c). The fibres were cellulosic.

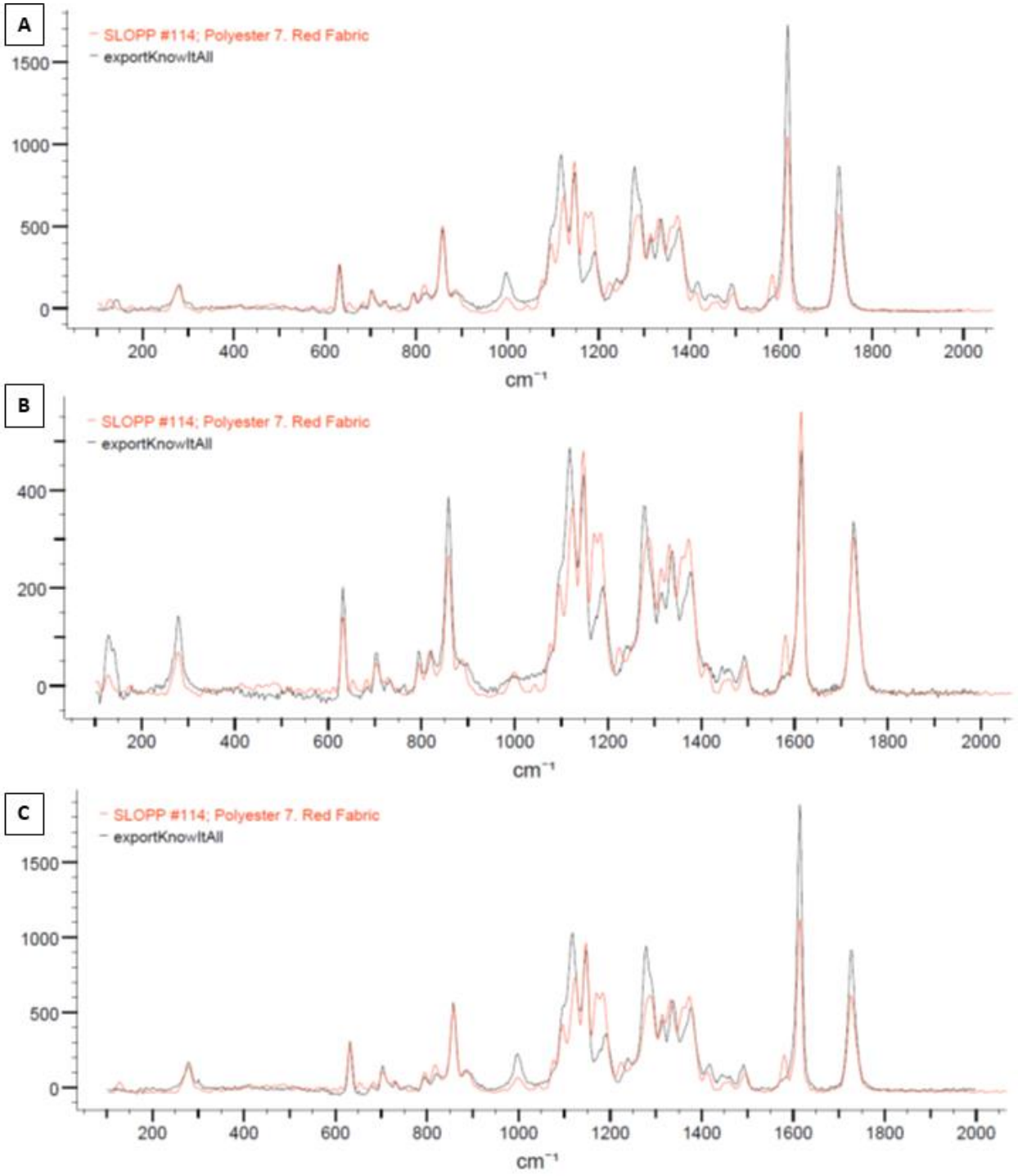


Figure S4. Fibres applied to a Skin Tac-coated PC filter using the proposed filtering technique were assessed using Raman spectroscopy (*N*=3; a, b, c). The fibres were identified as PET, and the filtering technique did not appear to interfere with polymer identification.

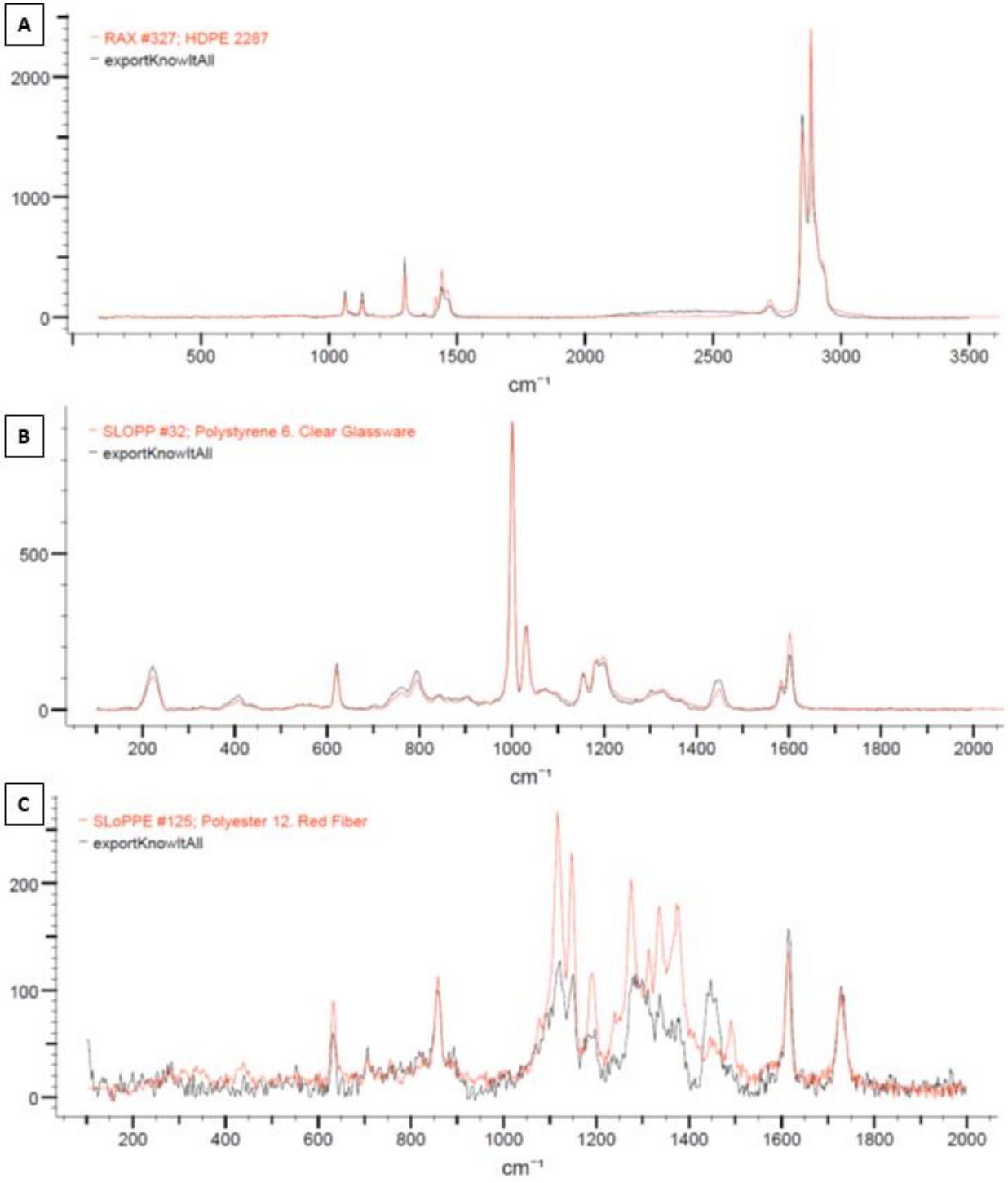
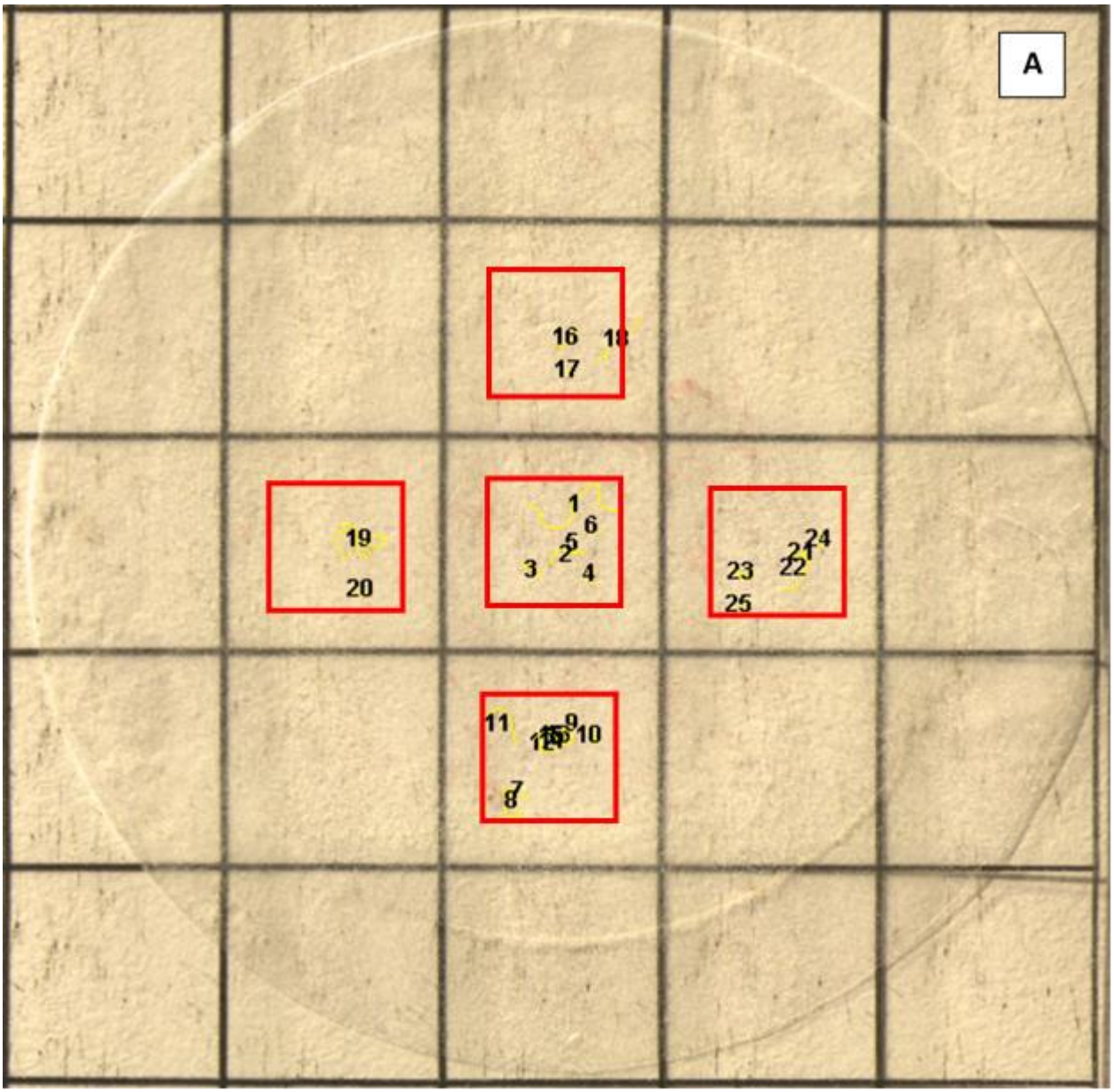
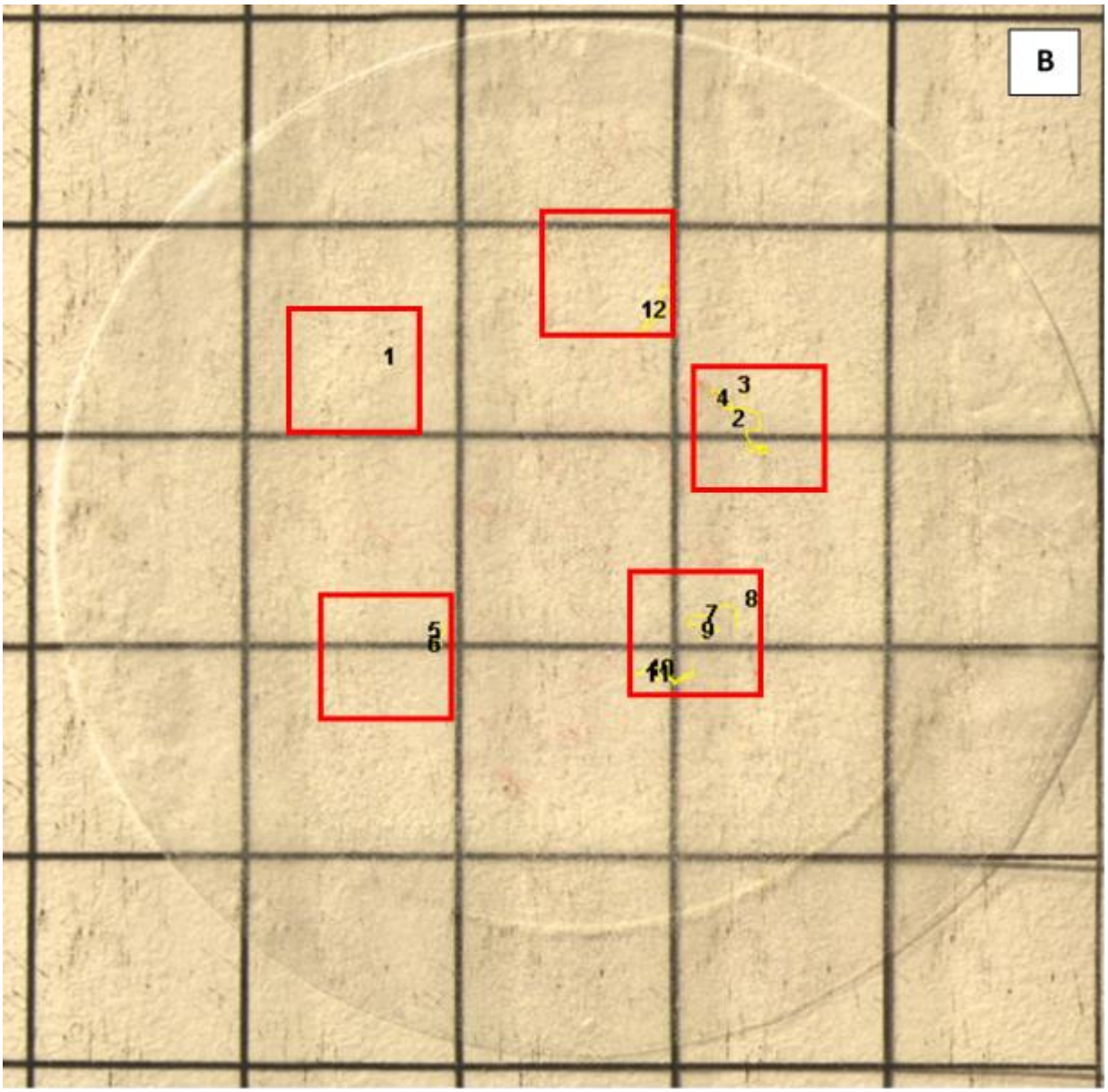
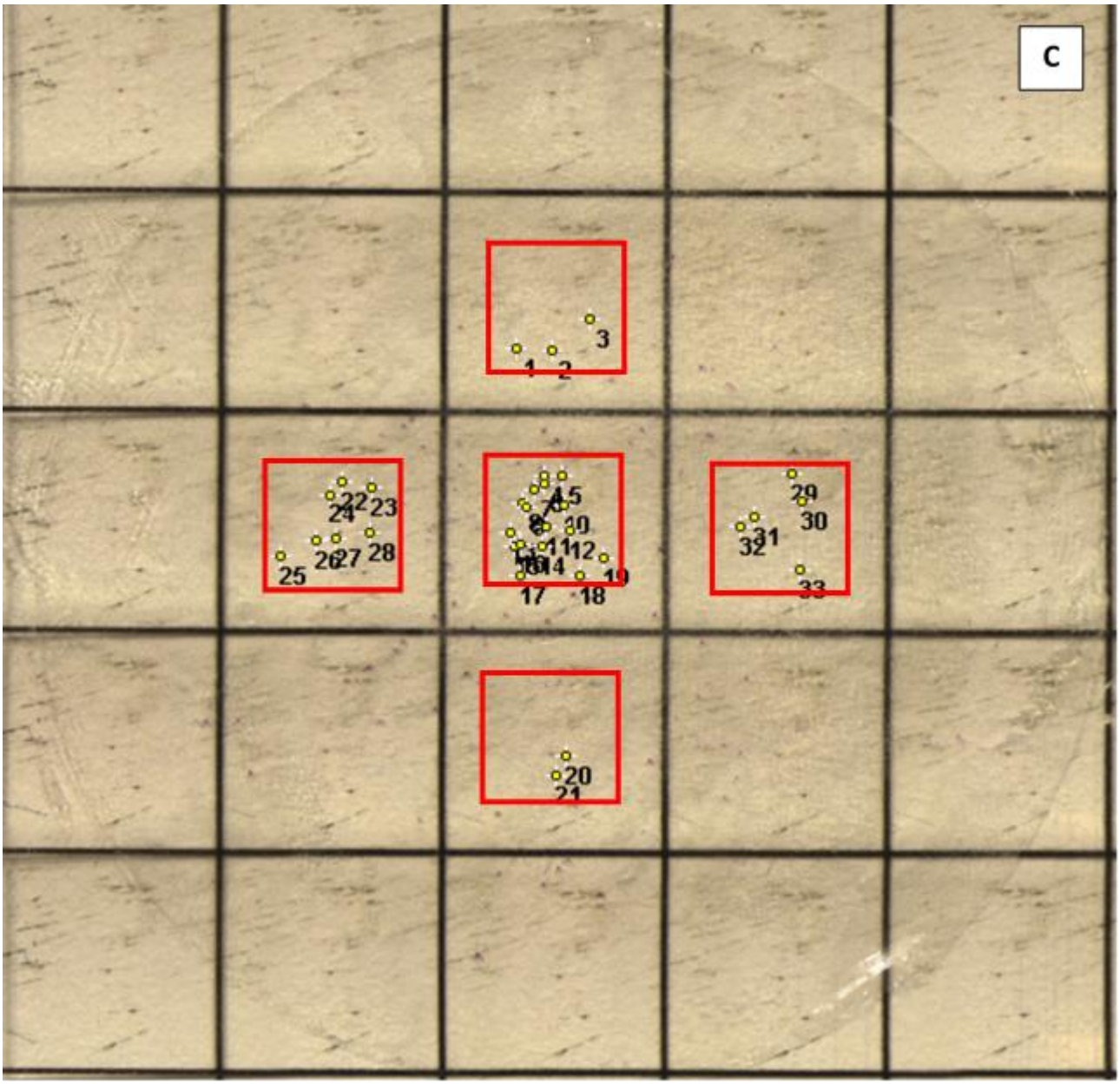


Figure S5. Fragments from a stock solution consisting of several common polymers were applied to a Skin Tac-coated PC filter using the proposed filtering technique and were assessed using Raman spectroscopy (*N*=3; a, b, c). The fragments were identified as PE (a), PS (b) and PET (c). The filtering technique did not appear to interfere with polymer identification.

Section 2: Landmarked filters for Method 1




B
12
1
4
3
2
8
7
9
5
6
10
11


C
3
1
2
22
23
24
25
26
27
28
15
30
11
12
14
19
17
18
29
30
31
32
33
20
21

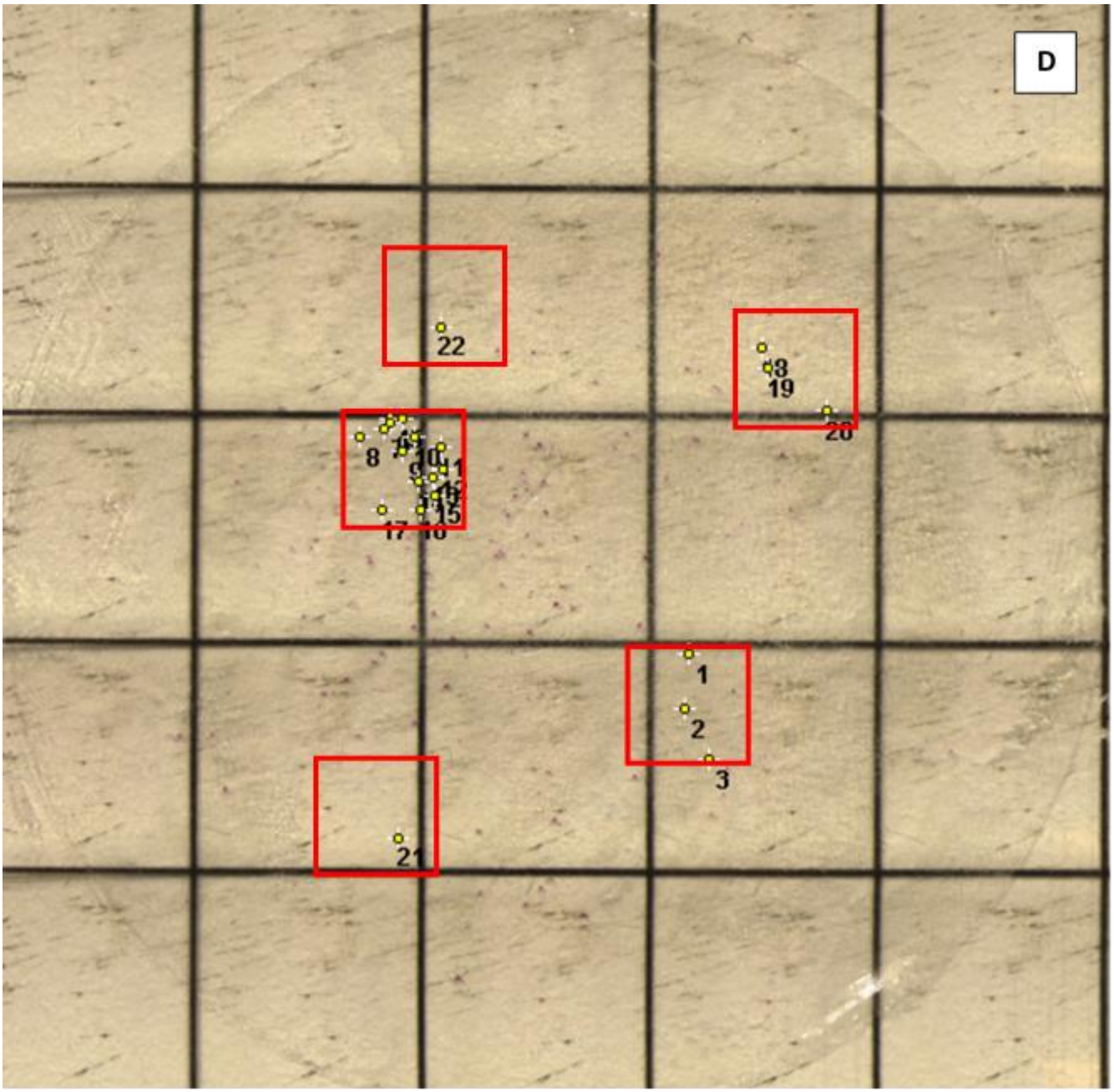


Figure S6. All particles within subsamples landmarked (location denoted by crosshairs). Subsample patterns include a) crosshair/line pattern for the red PET fibre filter (the line pattern is the three middle vertical squares; the crosshair pattern is all five squares) b) the random pattern for the red PET fibre filter c) crosshair/line pattern for the PVC/PET fragment mixture filter (the line pattern is the three middle vertical squares; the crosshair pattern is all five squares d) the random pattern for the PVC/PET fragment mixture filter.

Section 3: All 25 extrapolation trials and detailed instructions for extrapolation for Method 2; Landmarked filters for Method 2

Section 3a: *All 25 extrapolation trials and detailed instructions for extrapolation for Method 2*

After the filter was subdivided into concentric circles/rings (See Figure S7), the number of particles within each circle/ring was counted and the area of the circle/ring was calculated. The area of the subsampling square was calculated for each circle/ring — subsamples were 5 mm x 5 mm except for the inner circle which has a subsample area of 2.5 mm x 2.5 mm. A multiplication factor was then calculated by dividing the area of the subsample by the area of the circle/ring. Multiplication factors and how they were calculated are detailed in Table S4. After randomly selecting the subsample area within the circle, the number of particles were counted within the subsample. This particle number was then multiplied by the previously mentioned multiplication factor. This was repeated for each of the four concentric circles/ring. After an extrapolated particle number was determined for each concentric circle/ring, these numbers were added up to achieve a full filter extrapolated particle count. We did this for each concentric circle 25 times for both the fibre and fragment filter (Table S5).

Table S4. Multiplication factors used to extrapolate total particle counts for filters from Method 2. The area of the Inner Circle was calculated using the area of a circle formula (πr^2) where r (radius) for the Inner Circle = 5 mm. The areas of the Middle, Outer, and Exterior Rings were calculated by first calculating the area of a circle, where r = 10, 15, and 18 mm, for the Middle, Outer, and Exterior, respectively, and then subtracting the total area of the circle that preceded the current circle to get just the ring area. The multiplication factor for each circle or ring was then determined by dividing the area of the circle or ring by the area of the subsample. These multiplication factors can be used in conjunction with Table S2 to back calculate the original number of particles counted. Multiplication factors were the same for both the fibre and fragment filters as the areas were the same.

Circle or ring	*Area of circle or ring (mm^2)*	*Area of subsample (mm^2)*	***Multiplication factor (Area of circle or ring ⁄Area of subsample)***
Inner	25π	6.25	**4π**
Middle	75π	25	**3π**
Outer	125π	25	**5π**
Exterior	99π	25	$\frac{99}{25}\boldsymbol{\pi}$

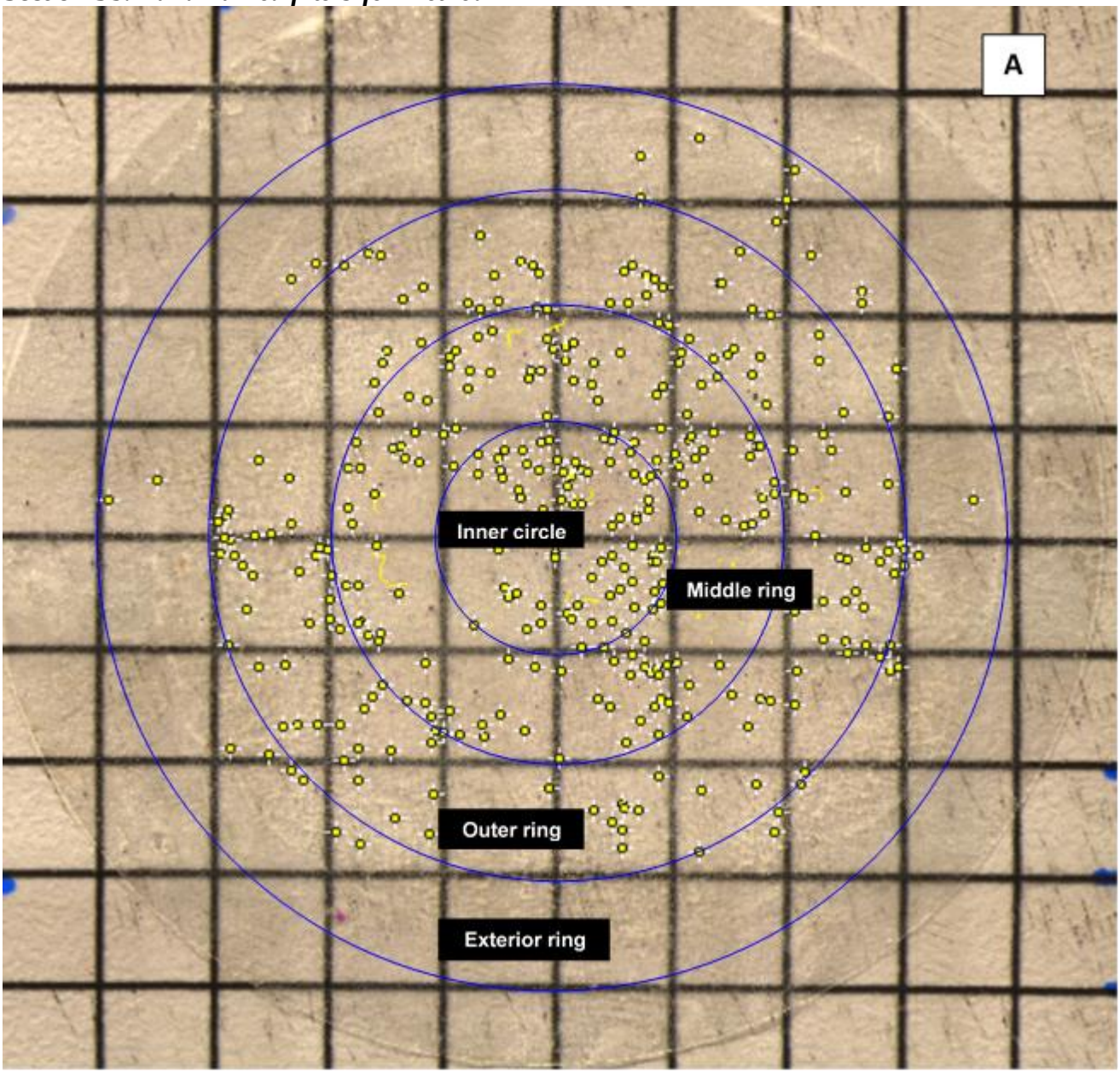
Table S5. All extrapolation counts for both the PET fibre filter and PVC/PET fragment mixture filter, including extrapolated counts based on subsampling and the sum of these extrapolated values. True particle count for red PET fibres filter is 376 and true particle count for the magenta PVC/PET mixture filter is 73. Note that some sums may be off by one particle compared to summing manually because of software rounding. This rounding discrepancy did not affect whether the total filter number was within a certain margin compared to summing manually (i.e. a one-particle discrepancy between summing manually or using software to sum did not change whether the total filter number was within 5%, 10%, or 20% of the true number).

RED PET FIBRES					
	Inner circle	*Middle ring*	*Outer ring*	*Exterior ring*	*Total filter (true number = 376)*
Extrapolated particle count based on one subsample per area	101	104	157	0	361 [a]
	38	113	110	50	311 [c]
	88	66	94	12	261 [x]
	101	141	110	0	352 [b]
	25	66	79	25	195 [x]
	50	66	94	0	210 [x]
	38	151	110	25	323 [c]
	25	104	173	12	314 [c]
	38	113	47	25	223 [x]
	25	66	16	0	107 [x]
	101	141	126	0	368 [a]
	13	170	126	37	345 [b]
	75	113	79	12	279 [x]
	101	113	63	0	276 [x]
	63	132	16	37	248 [x]
	75	141	47	0	264 [x]
	63	66	94	0	223 [x]
	101	75	79	0	254 [x]
	13	113	126	37	289 [x]
	38	132	63	50	282 [x]

	101	132	236	0	468 [x]
	63	57	94	25	239 [x]
	50	151	157	12	371 [a]
	63	66	204	12	345 [b]
	63	94	173	0	330 [c]
MAGENTA PVC/PET FRAGMENT MIXTURE					
	Inner circle	*Middle ring*	*Outer ring*	*Exterior ring*	*Total filter (true number = 73)*
Extrapolated particle count based on one subsample per area	25	94	0	0	119 [x]
	38	19	16	0	72 [a]
	13	9	16	0	38 [x]
	38	0	47	12	97 [x]
	50	28	0	12	91 [x]
	13	38	31	0	82 [c]
	25	19	16	0	60 [x]
	38	9	0	0	47 [x]
	13	0	16	0	28 [x]
	0	28	0	0	28 [x]
	13	0	0	12	25 [x]
	38	57	0	0	94 [x]
	38	57	0	0	94 [x]
	38	0	16	12	66 [c]
	0	0	16	0	16 [x]
	0	0	47	0	47 [x]
	13	19	0	0	31 [x]
	0	9	47	0	57 [x]
	38	28	16	12	94 [x]
	13	19	16	0	47 [x]
	13	28	16	0	57 [x]
	13	113	16	0	141 [x]
	13	0	0	0	13 [x]
	101	19	16	0	135 [x]

[a] Indicates extrapolated value that's within ±5% of the true particle count, [b] indicates extrapolated value that's within ±10% of the true particle count, [c] indicates extrapolated value that's within ±20% of the true particle count, [x] Indicates extrapolated value that's outside ± 20% of the true particle count.

Section 3b*: Landmarked filters for Method 2*



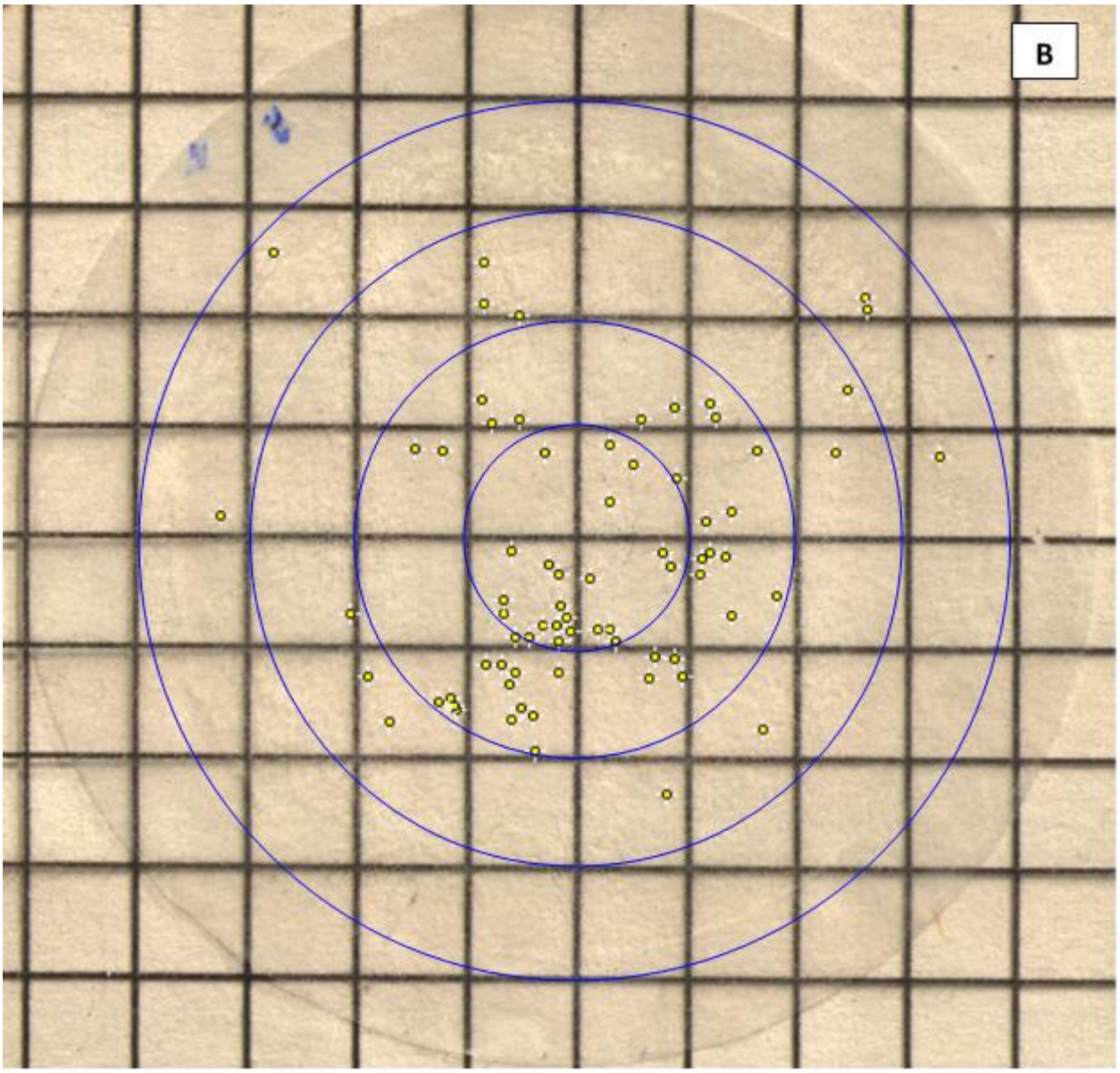
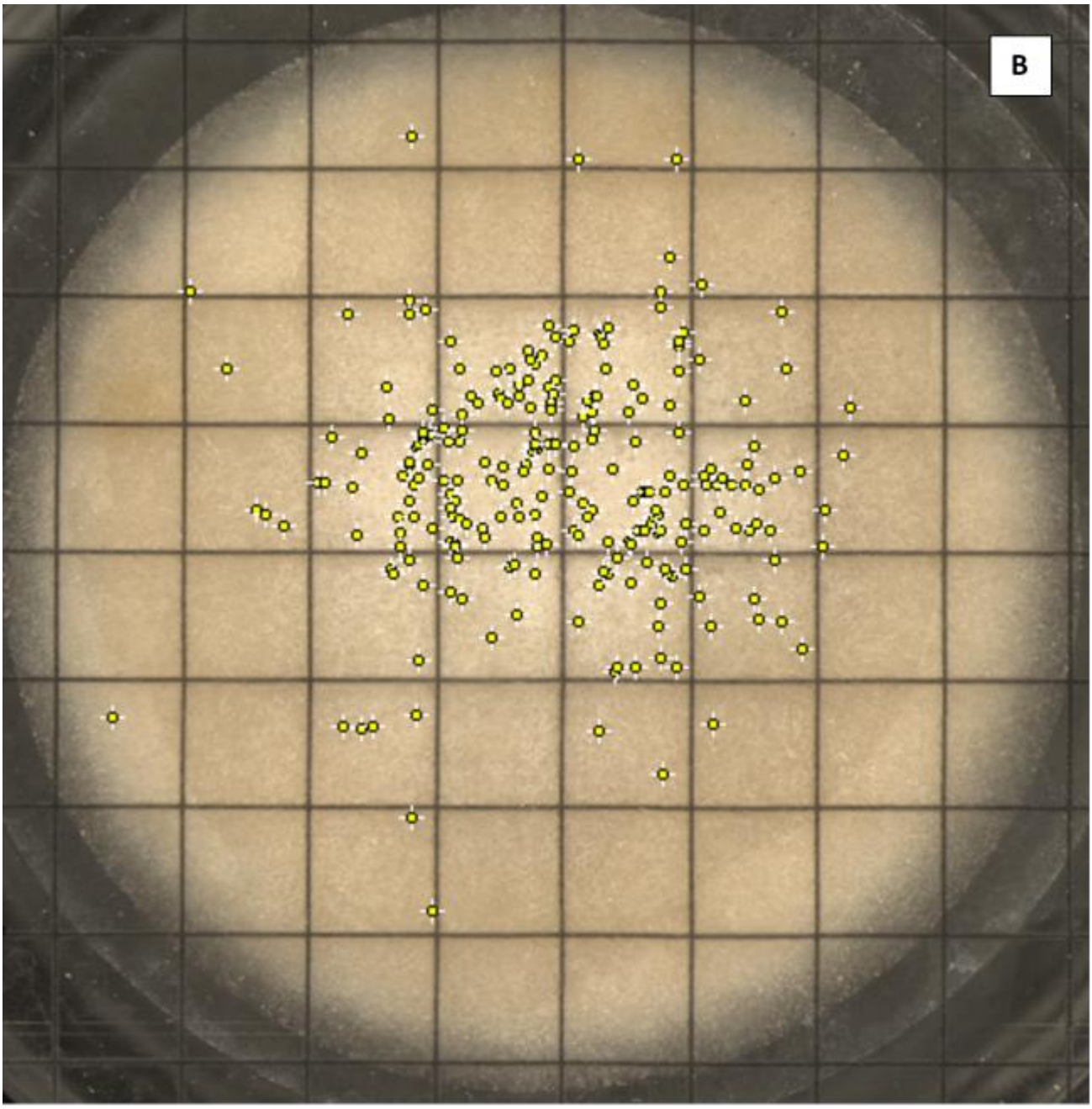
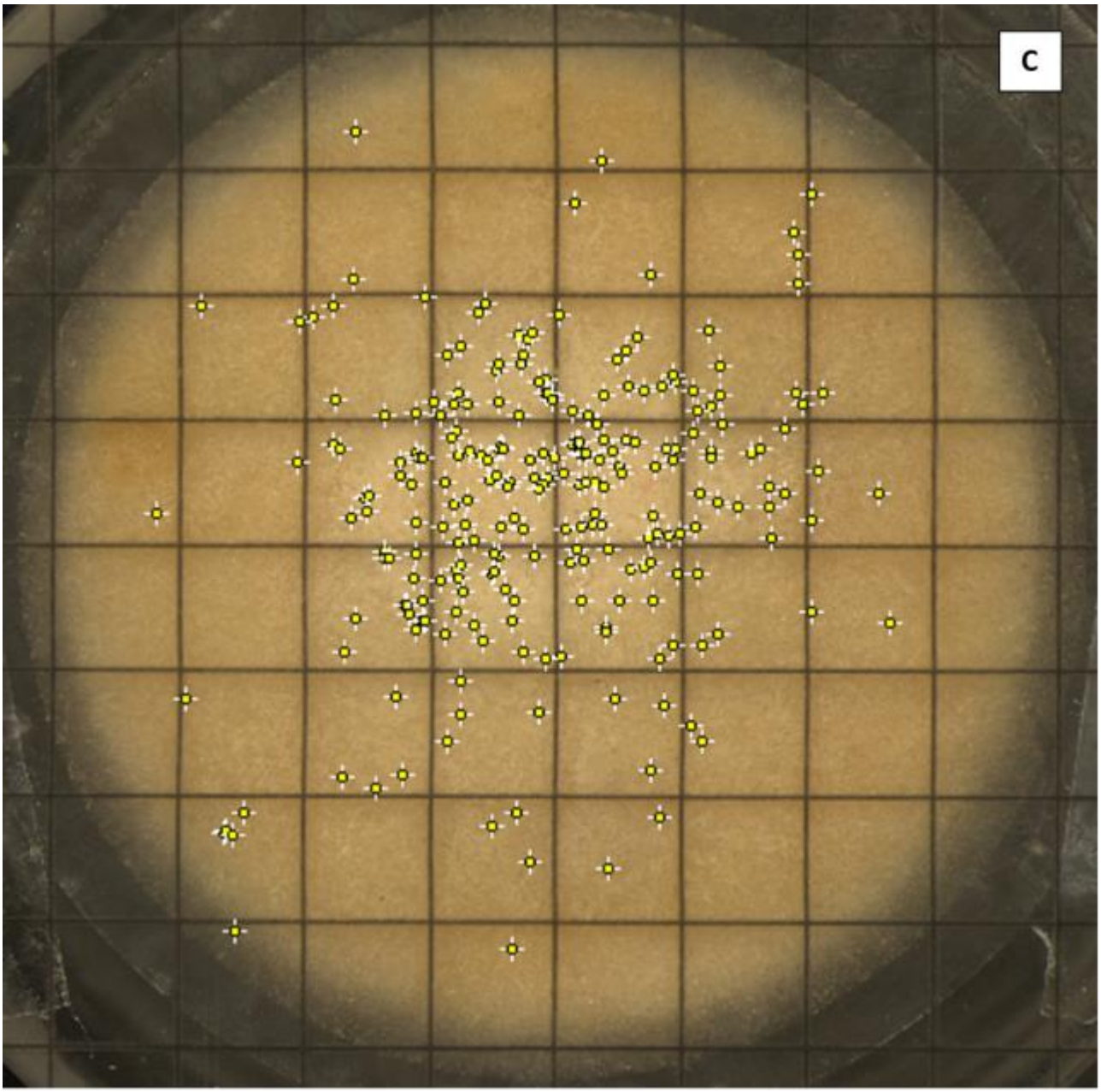
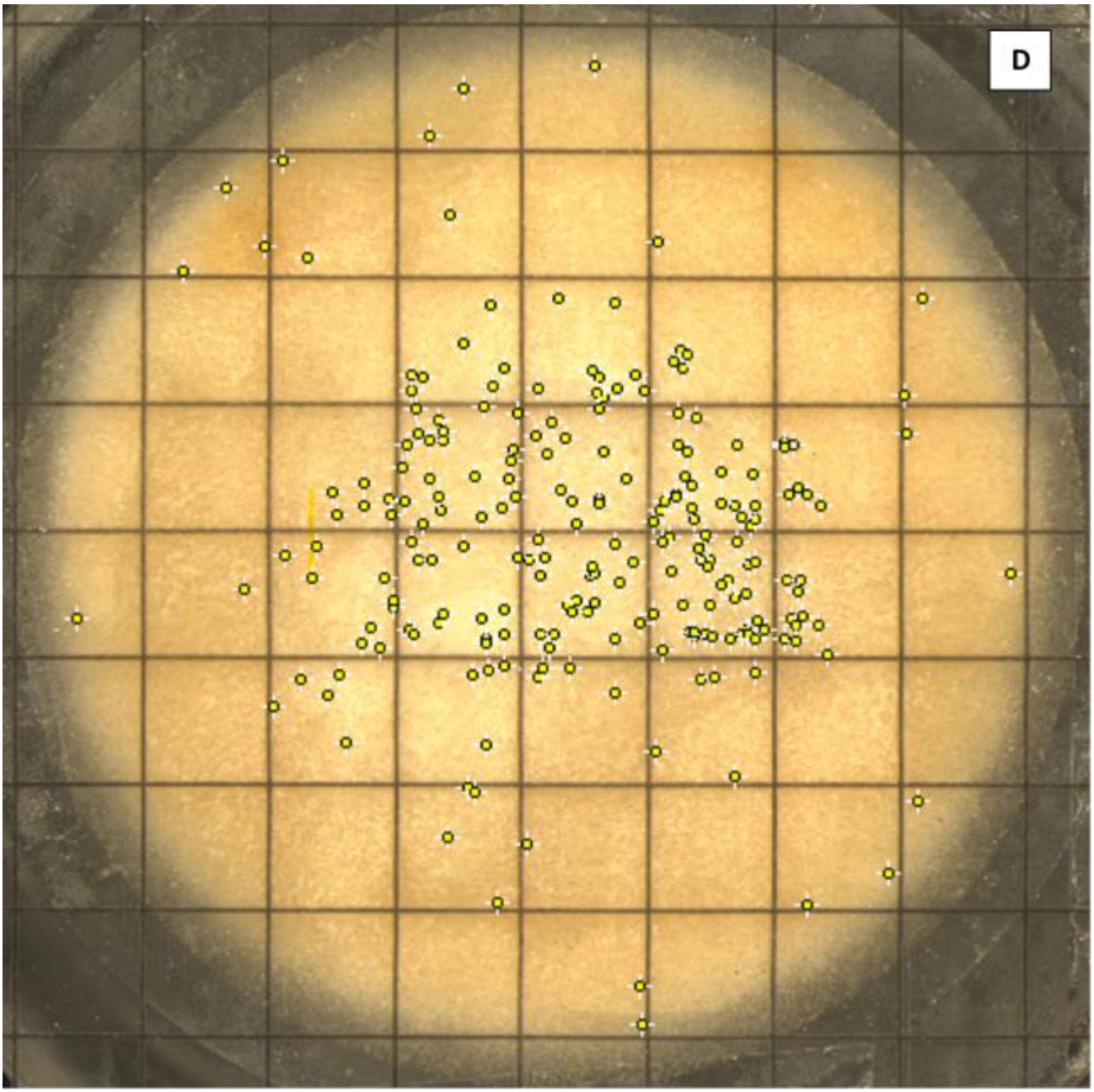


Figure S7. The a) red PET fibre and b) magenta PVC/PET fragment mixture filters used in the concentric circle analysis with all particles landmarked (particle location denoted by crosshairs). The subdivided concentric circles are designated by the blue circles. A randomly placed 5 mm x 5 mm square subsample was taken from each circle, except for the inner most circle where a 2.5 mm x 2.5 mm square subsample was taken. The names of the circles and rings denoted on a) apply to b) as well.

Section 4: Landmarked filters for Method 3


B


C


D

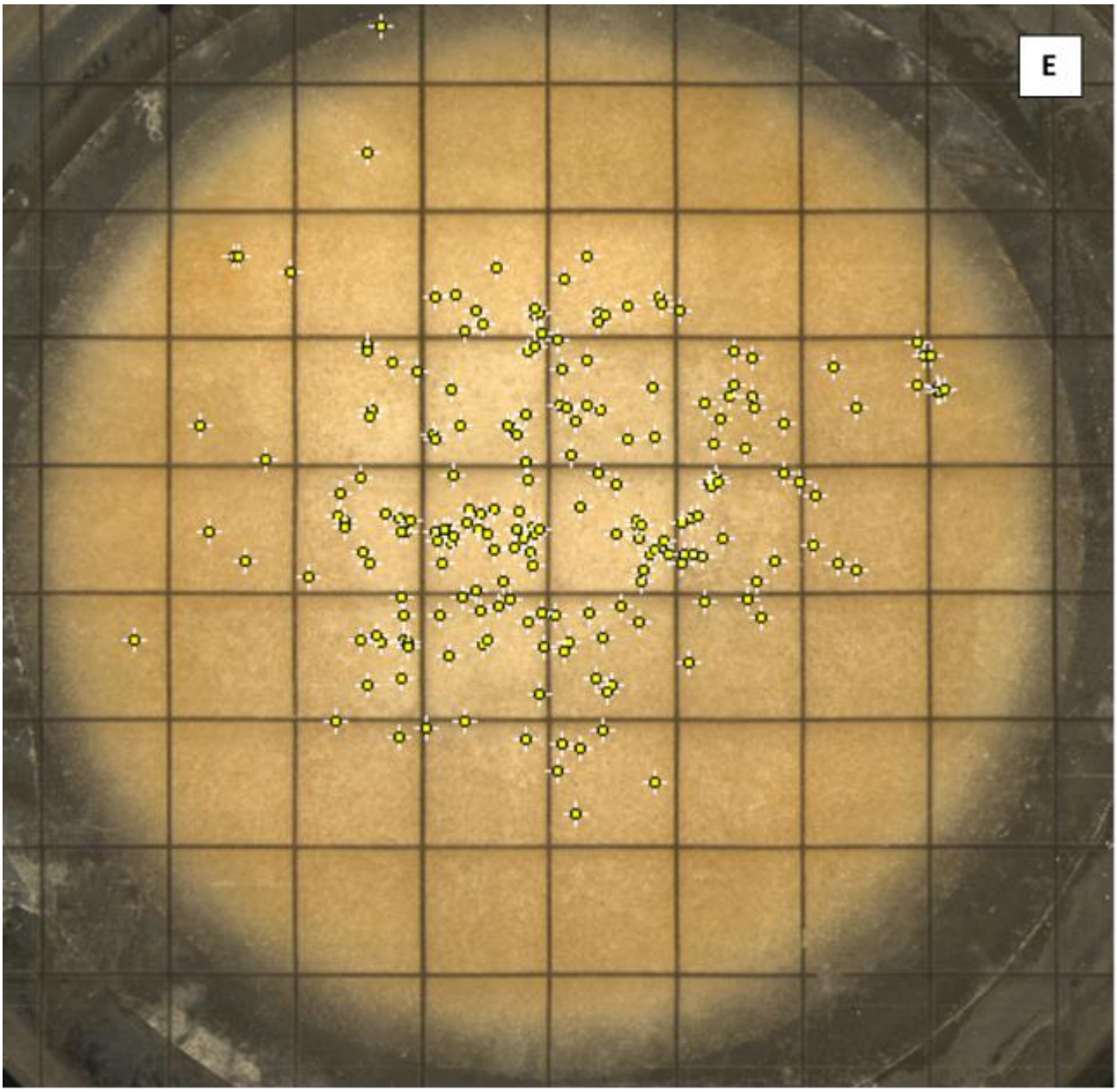
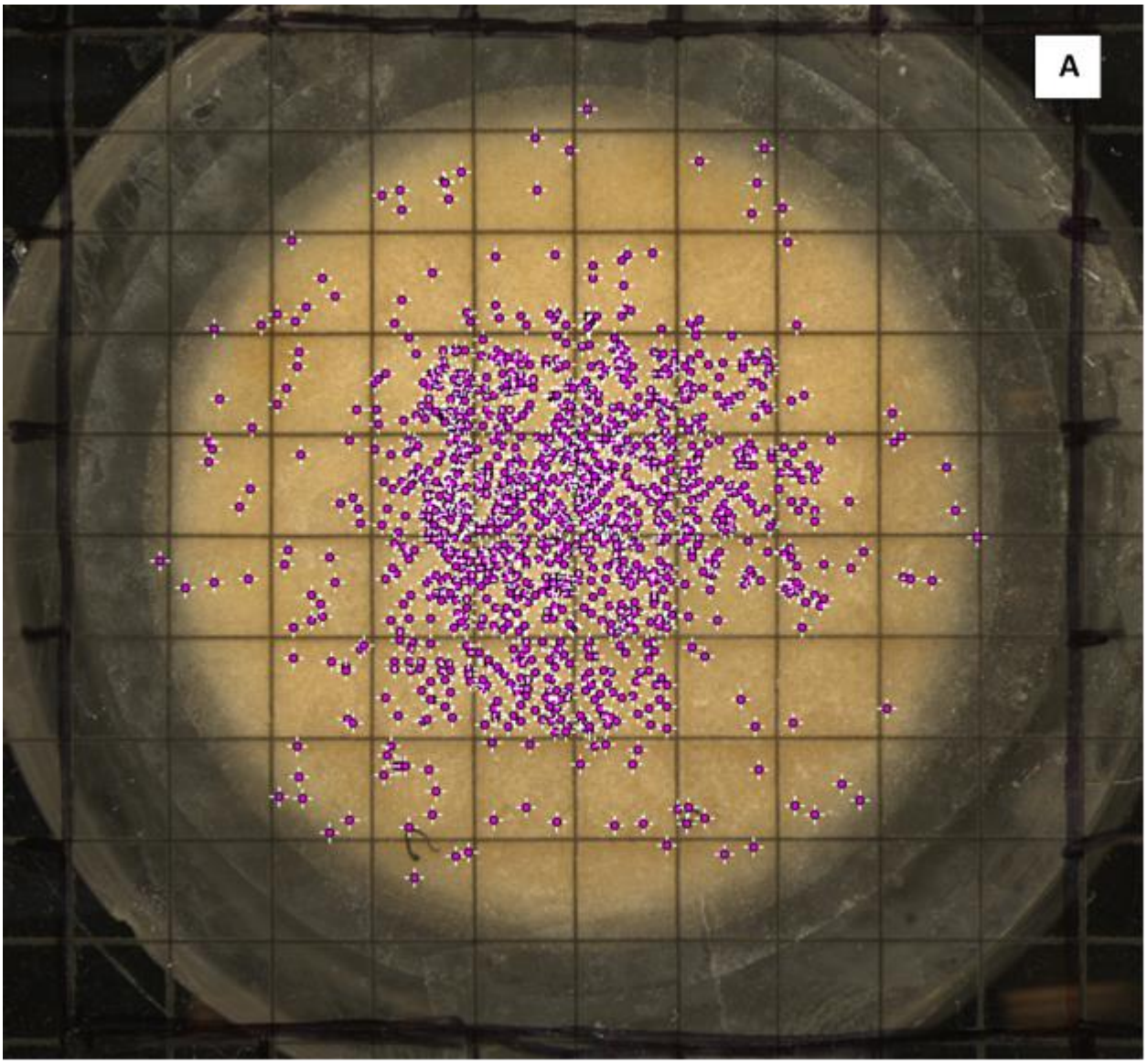
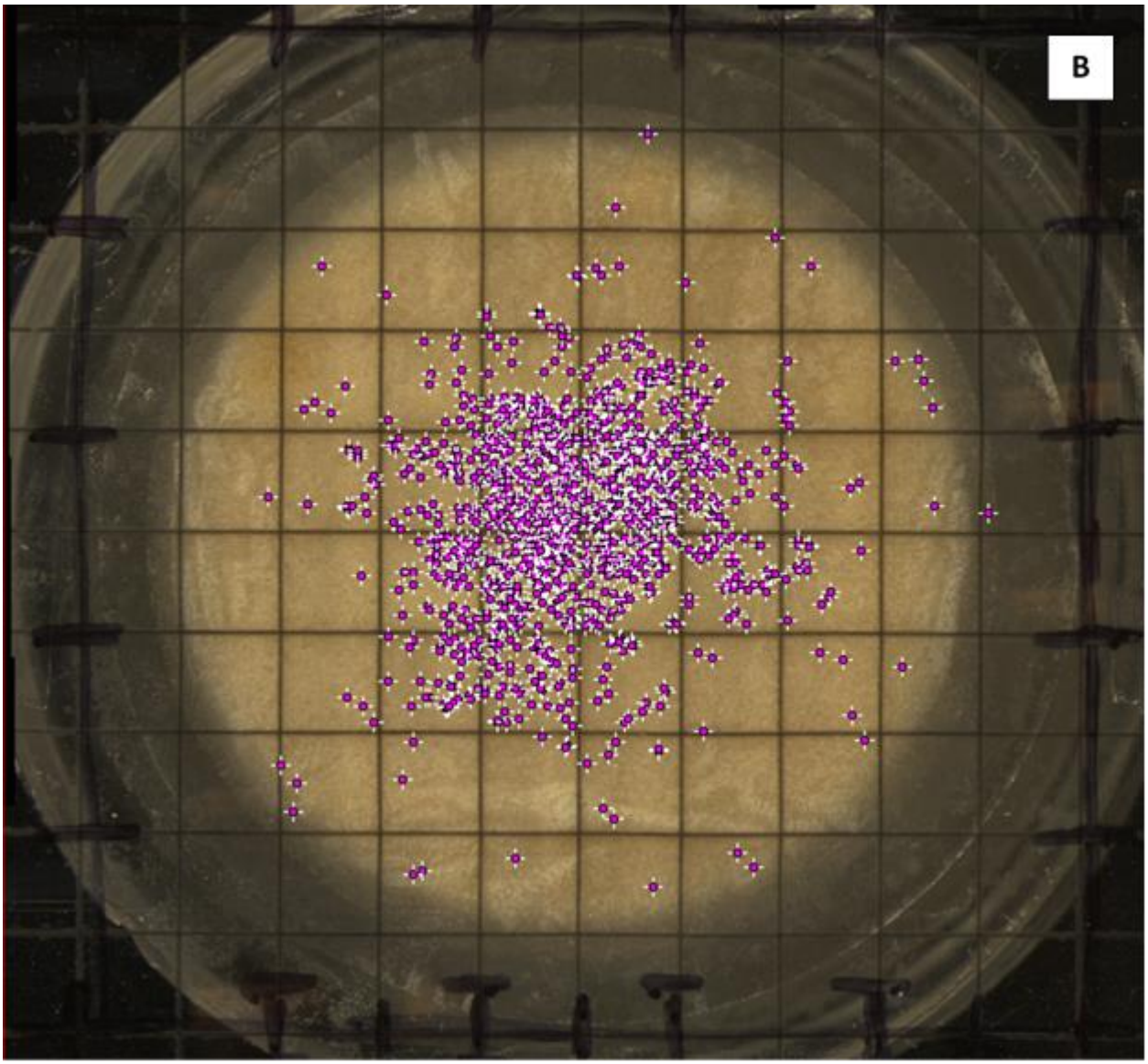


Figure S8. Particles from laboratory-prepared spiked plastic solution. Location of particles denoted by crosshairs. Filter preparation methods are described in Table 1 in the main text. All filters were analyzed using Method 3. Particles were landmarked using ImageJ (version 1.52n, National Institutes of Health). (a=Filter 1; b=Filter 2; c=Filter 3; d=Filter 4; e=Filter 5).


A


B

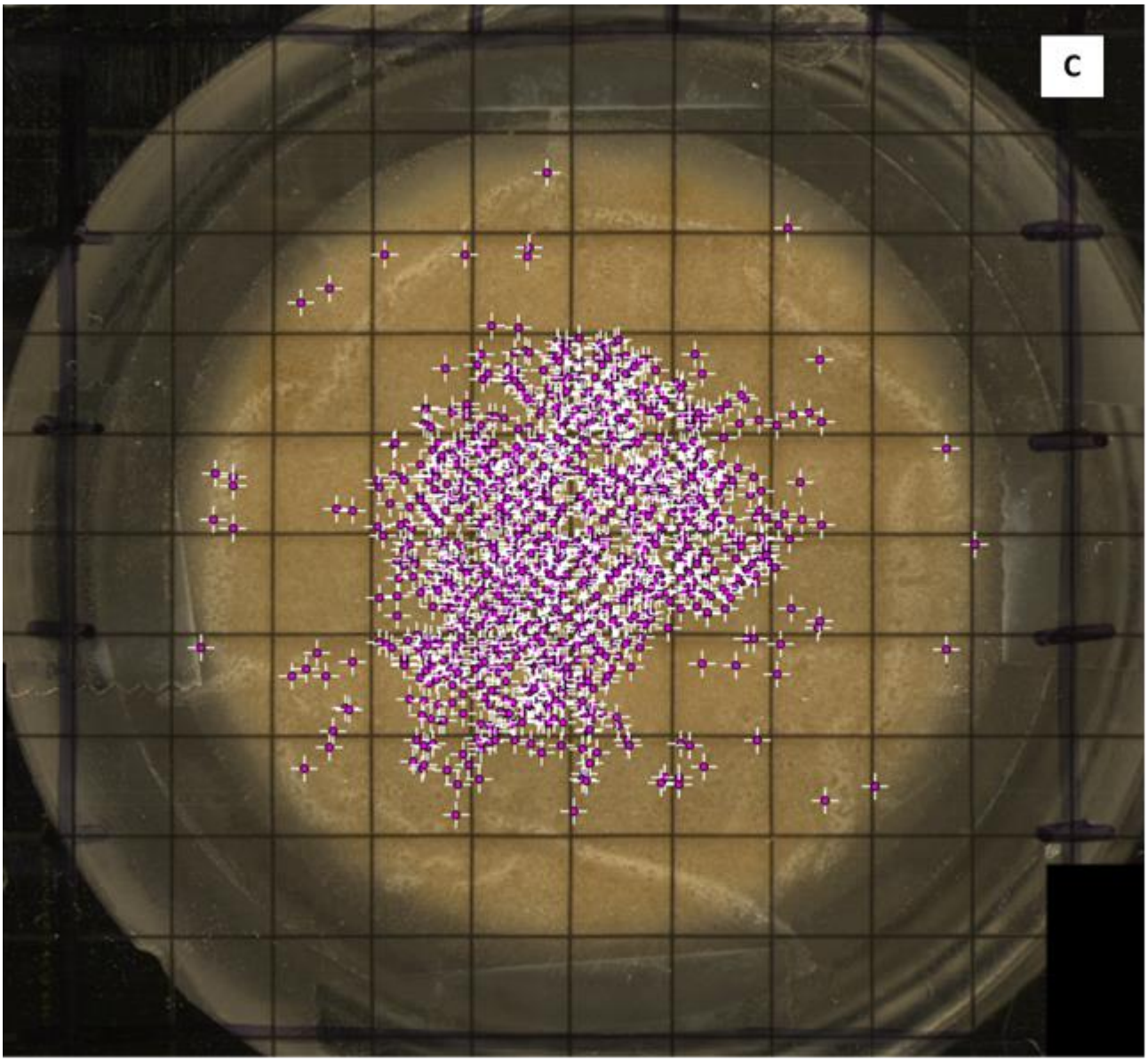


Figure S9. Particles from 20 L of filtered laboratory tap water. Location of particles denoted by crosshairs. Filter preparation methods are described in Table 1 of the main text. All filters were analyzed using Method 3. Particles were landmarked using ImageJ (version 1.52n, National Institutes of Health). (a=Filter 1; b=Filter 2, c=Filter 3).