

## **Supplementary material:**

# **Fast Forward: Optimized Sample Preparation and Fluorescent Staining for Microplastic Detection**

**Michael Toni Sturm <sup>1</sup>, Erika Myers <sup>1</sup>, Anika Korzin <sup>1,2</sup>, Sabrina Polierer <sup>1,2</sup> Dennis Schober <sup>1,2</sup>, and  
Katrin Schuhen<sup>1,\*</sup>**

<sup>1</sup> Wasser 3.0 gGmbH, Neufeldstr. 17a-19a, 76187 Karlsruhe, Germany; [info@wasserdreinull.de](mailto:info@wasserdreinull.de)

<sup>2</sup> abcr GmbH, Im Schleht 10, 76187 Karlsruhe, Germany; [info@abcr.com](mailto:info@abcr.com)

\* Correspondence: [schuhen@wasserdreinull.de](mailto:schuhen@wasserdreinull.de), +49 721 15 65 95 93

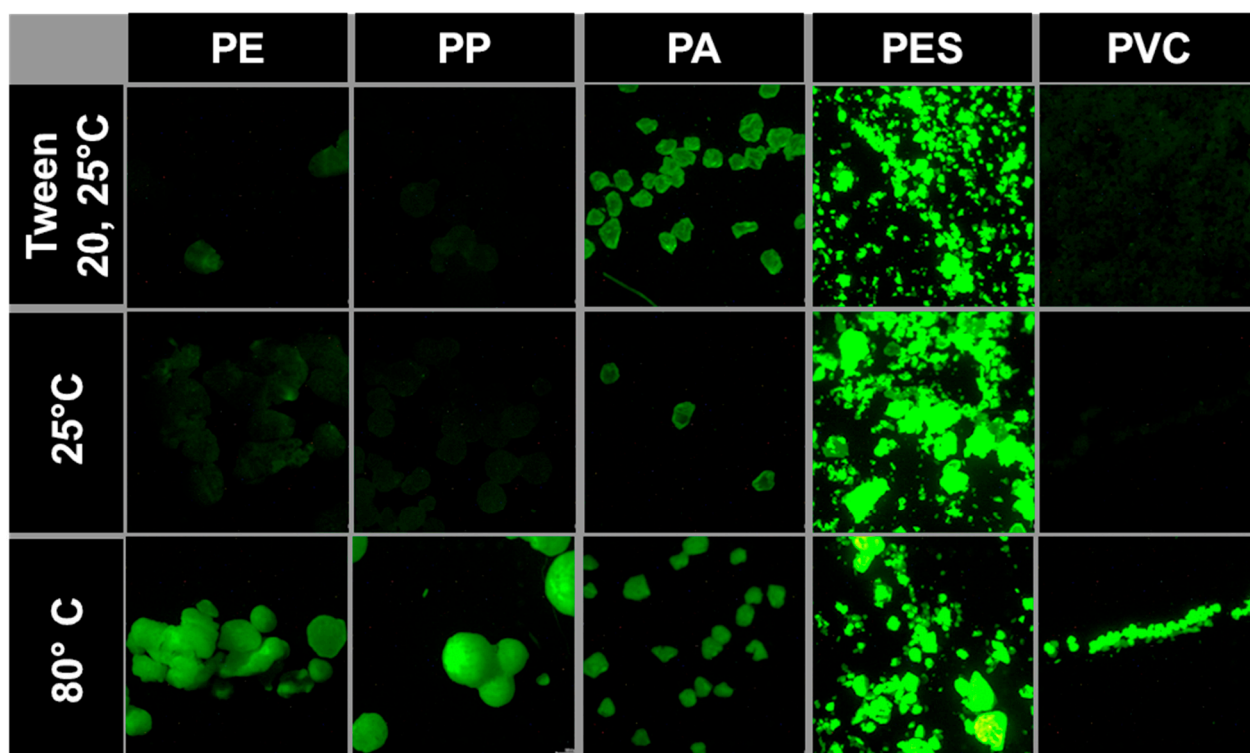


Figure S1: Surfactant (Tween 20) as staining aid – fluorescent images of microplastics from different polymers stained with 0.5 mg / l abcr eco Wasser 3.0 detect mix MP-1 for 1 hour with and without Tween 20 (0.5 mg/l).

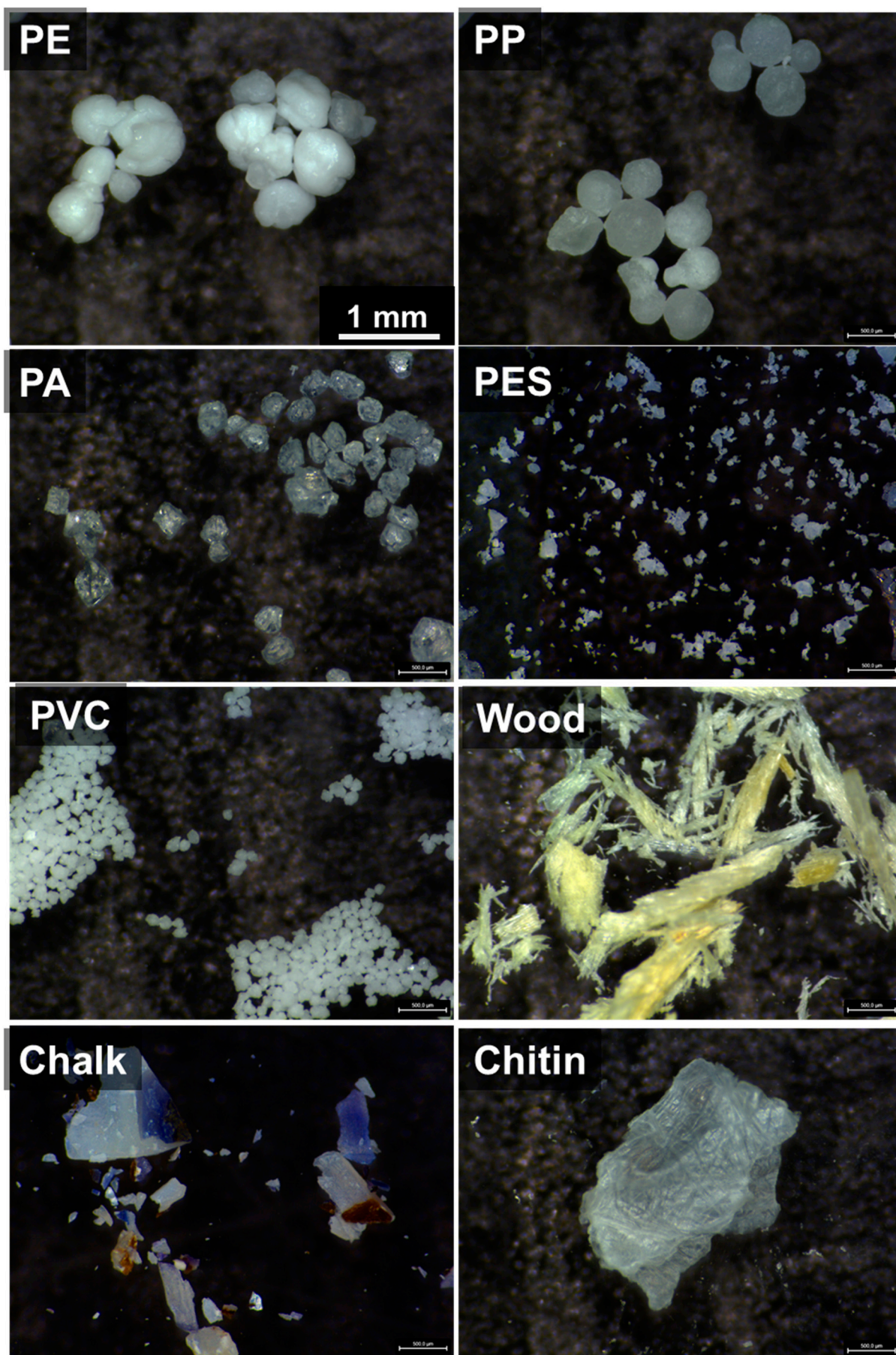


Figure S2: Fotos of the microplastics (PE, PP, PA, PES, PVC) and natural particles (wood, chalk, chitin) used for the recovery rates.

*Table S1: Measurement data for the recovery rates for microplastics and natural particles using the slow hydrogen peroxide digestion (4 h 80 °C, 20 h R.T.)*

<b>Particle / Run no.</b>	<b>Detected particles</b>	<b>Recovery [%]</b>	<b>Mean [%]</b>	<b>S.D. [%]</b>
PE 1	38	95	95.8	1.2
PE 2	39	97.5		
PE 3	38	95		
PP 1	42	105	92.5	8.9
PP 2	34	85		
PP 3	35	87.5		
PA 1	39	97.5	101.7	3.1
PA 2	42	105		
PA 3	41	102.5		
PES 1	42	105	100.8	4.2
PES 2	38	95		
PES 3	41	102.5		
PVC 1	41	102.5	100.0	2.0
PVC 2	39	97.5		
PVC 3	40	100		
Wood 1	4	10	5.8	3.1
Wood 2	1	2.5		
Wood 3	2	5		
Chalk 1	1	2.5	13.3	9.2
Chalk 2	5	12.5		
Chalk 3	10	25		
Chitin 1	0	0	2.5	2.0
Chitin 2	1	2.5		
Chitin 3	2	5		

Table S2 Measurement data for the recovery rates for microplastics and natural particles using the fast hydrogen peroxide digestion (1 h 100 °C)

Particle / Run no.	Detected particles	Recovery [%]	Mean [%]	S.D. [%]
PE 1	41	102.5	97.5	3.5
PE 2	38	95		
PE 3	38	95		
PP 1	36	90	93.3	4.7
PP 2	40	100		
PP 3	36	90		
PA 1	41	102.5	101.7	1.2
PA 2	40	100		
PA 3	41	102.5		
PES 1	42	105	100.0	5.4
PES 2	37	92.5		
PES 3	41	102.5		
PVC 1	37	92.5	96.7	3.1
PVC 2	39	97.5		
PVC 3	40	100		
Wood 1	1	2.5	5.0	3.5
Wood 2	4	10		
Wood 3	1	2.5		
Chalk 1	3	7.5	5.0	3.5
Chalk 2	0	0		
Chalk 3	3	7.5		
Chitin 1	0	3	5.8	1.2
Chitin 2	1	2		
Chitin 3	2	2		



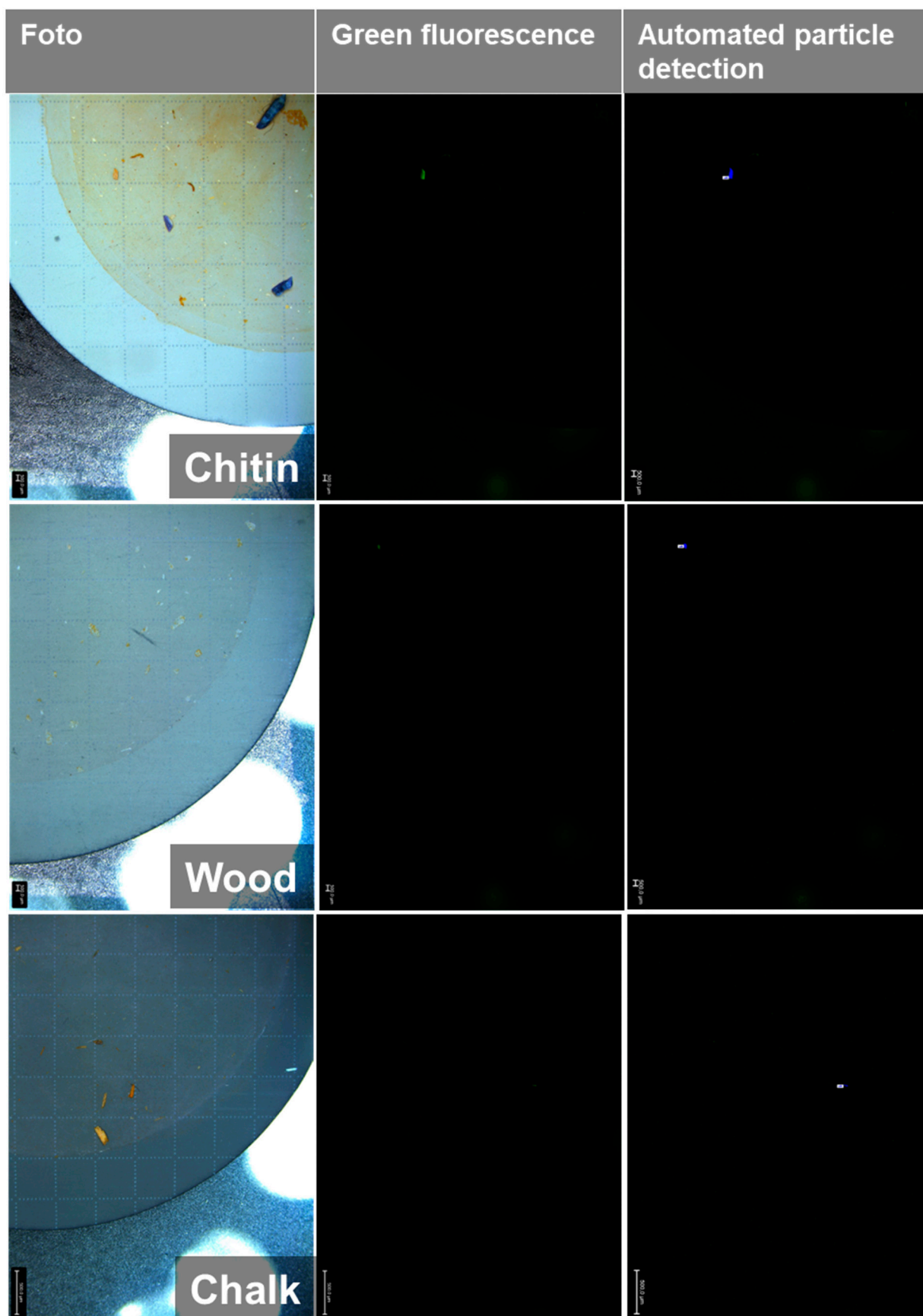


Figure S3.1: Caption - see Fig. S3.3

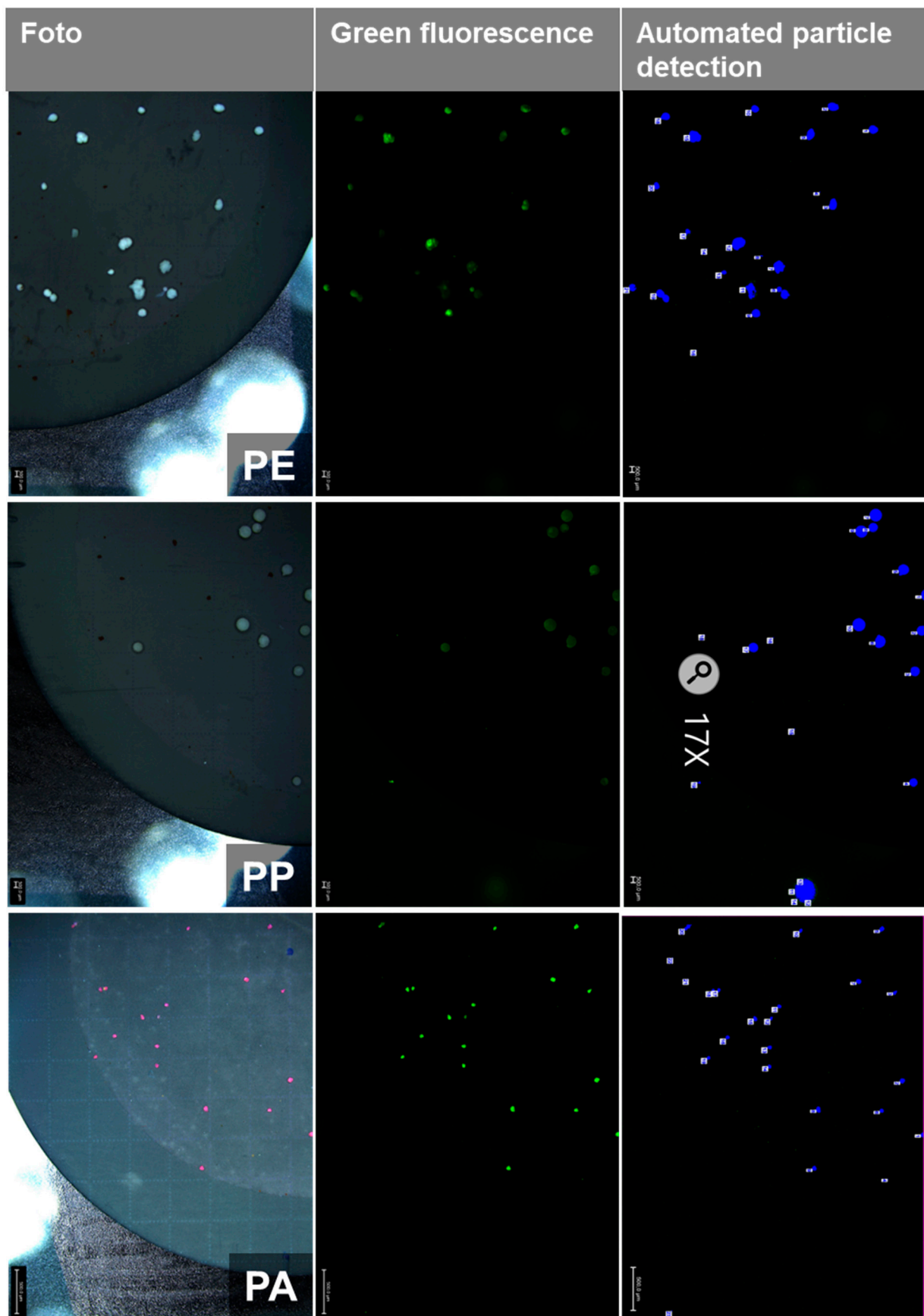


Figure S3.2: Caption - see Fig. S3.3

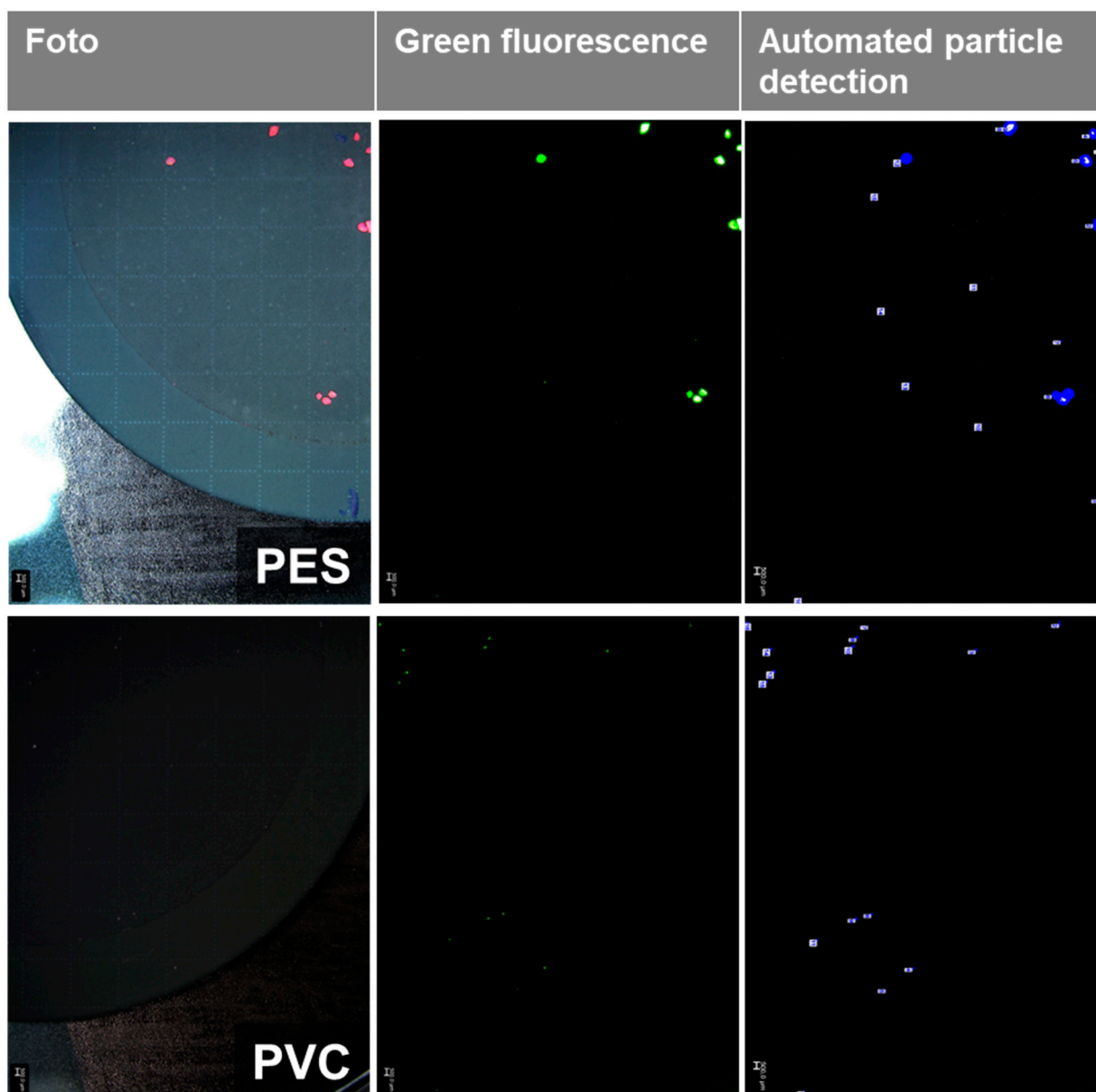


Figure S3.3: Recovery rate experiment images using the slow hydrogen peroxide digestion (4 h 80 °C, 20 h R.T.) - Photos, green fluorescent images and images from automated particle detection of subsamples for the recovery rates using the slow hydrogen peroxide digestion (4 h 80 °C, 20 h R.T.) and fluorescent staining with 0.25 mg/l MP-1 for 1h at 80°C.

The images of the samples were taken after the hydrogen peroxide treatment, fluorescent staining, and filtration on the black filter membranes. Due to the limited object field, every sample (respective filter) was split in four images. Here one image of the sample is displayed.

For the microplastics particles, optically no harm can be detected (compare Fig. S2). Chalk also remains unaffected by the hydrogen peroxide treatment; wood is partially degraded, and chitin shows strong fragmentation. As shown in Sturm et al. (2022) the hydrogen peroxide treatment reduces the fluorescent signal of the natural particles strongly and reduces the risk of false positives.

For the automated particle detection, no image processing filters were applied. The brightness threshold was set to 25 (brightness range from 0-255). Blue marked areas are detected and counted as microplastics. False positives by reflections (see PP) were subtracted from the particle count. Its notable, that the algorithm has problems with touching particles, which are detected as one. Applying a split touch filter leads to many false positives by splitting random particles with the used software (LAS-X 3.0.1423224), which is why it was not used. This is especially problematic in samples with high particle count. Further, there is a variance of brightness within the particles itself, which is why the algorithm only detects some of them partially. Application of binary filters as opening, this problem could be reduced, but the problem of touching particles being detected as one particle was enhanced. Therefore no binary filter was used.



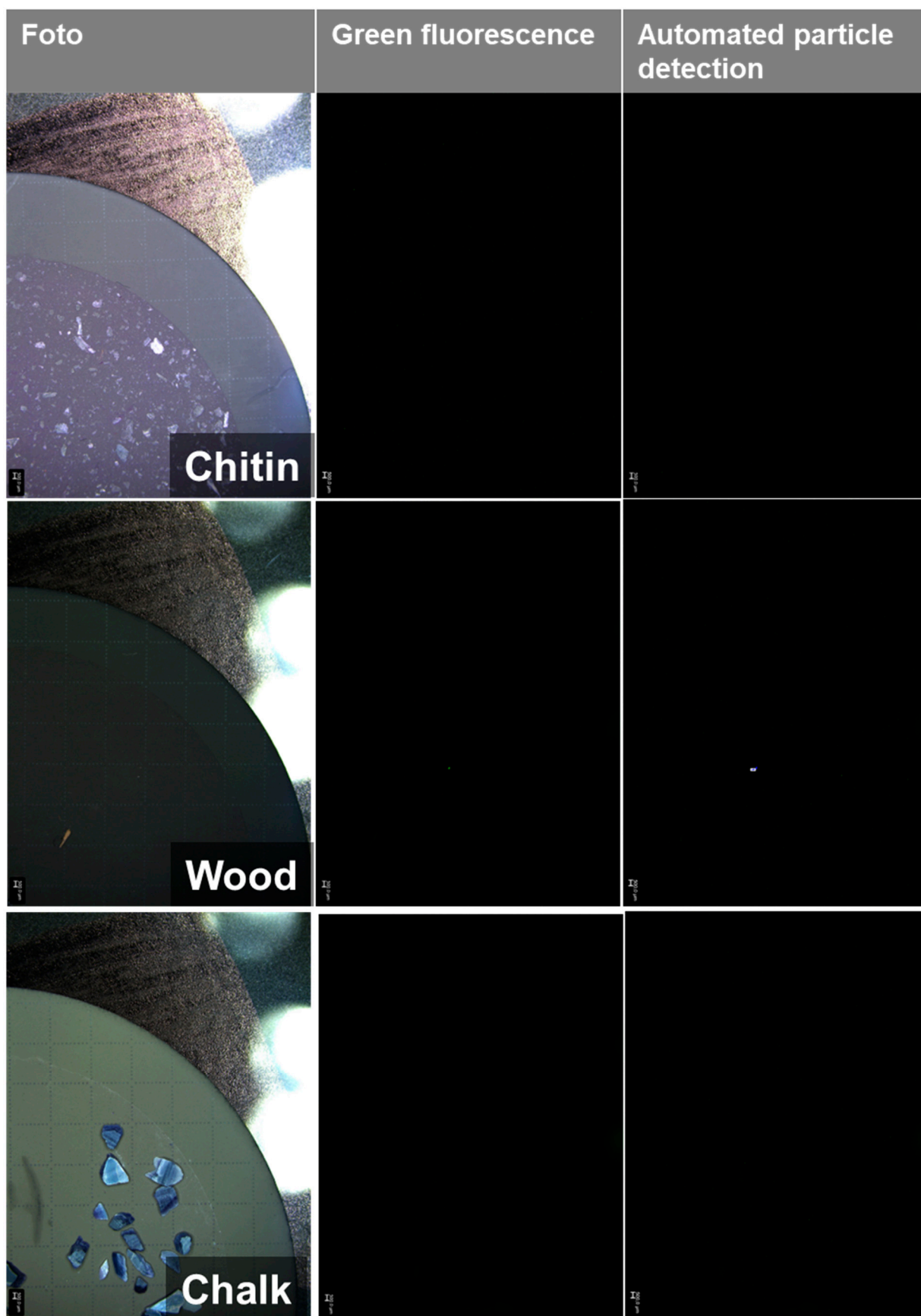


Figure S4.1: Caption - see Fig. S4.3

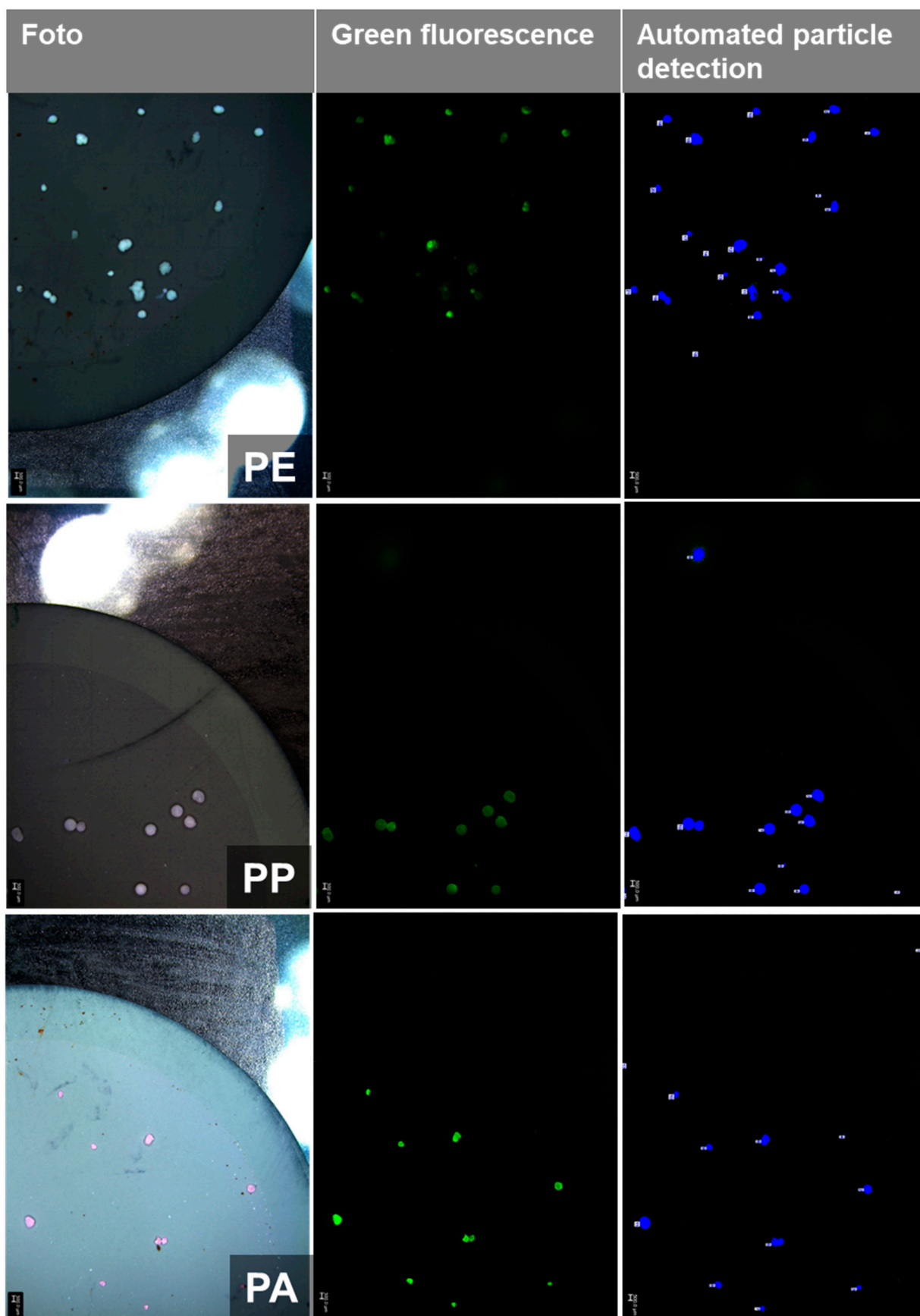


Figure S4.2: Caption - see Fig. S4.3

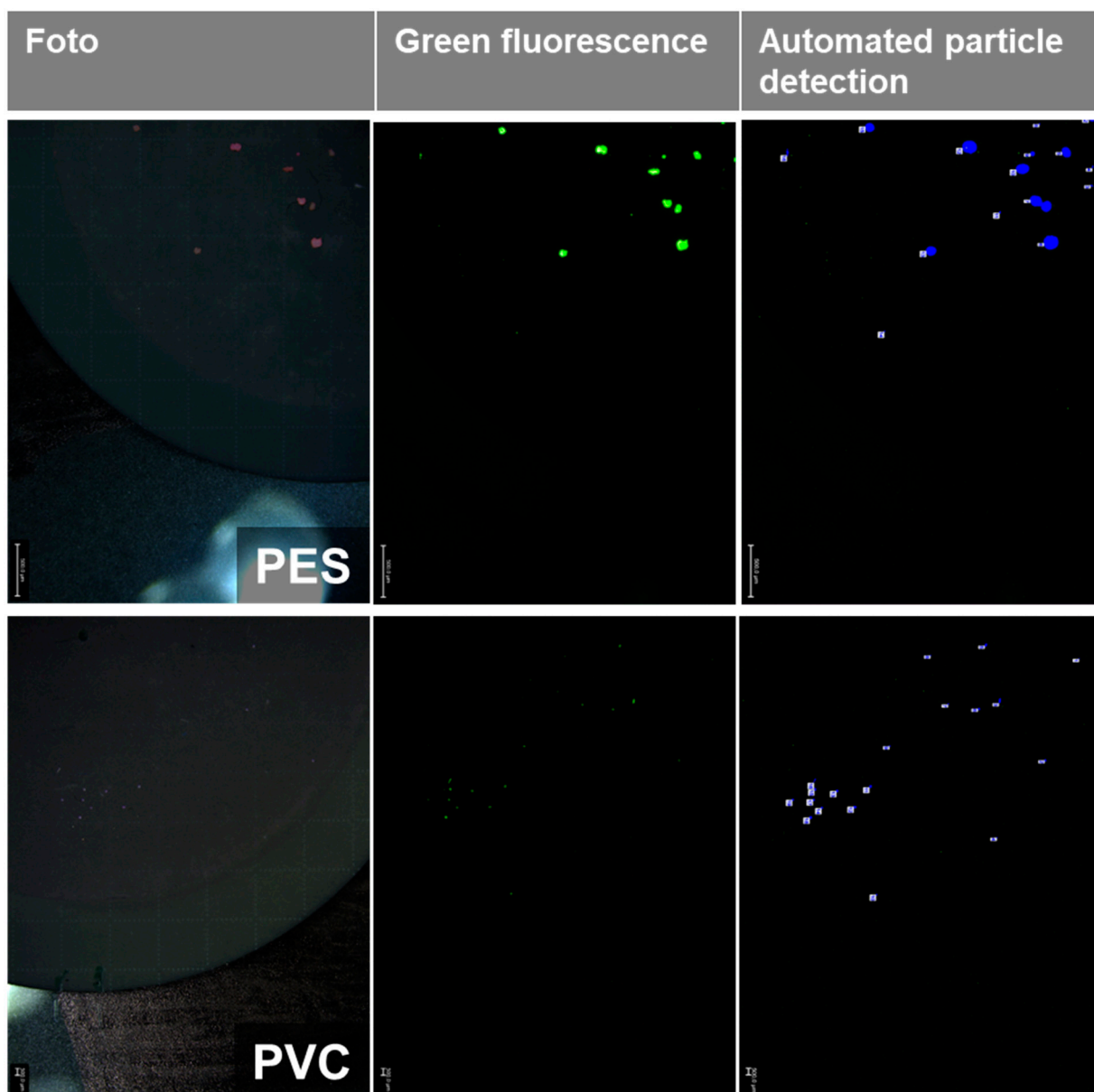


Figure S4.3: Recovery rate experiment images using the fast hydrogen peroxide digestion (1h at 100°C) - Photos, green fluorescent images and images from automated particle detection of subsamples for the recovery rates using the fast hydrogen peroxide digestion (1h at 100°C) and fluorescent staining with 0.25 mg/l MP-1 for 1h at 80°C.

Compared to the slow hydrogen peroxide digestion, no differences are visible by optical evaluation. Thus, the samples can be treated efficiently with a reduced processing time.

*Table S3: Measured microplastics contamination and deviations of the wastewater samples from the effluent of the WWTP Landau. The same samples were measured with the fast method based on the current paper and the slow method based on Sturm et al. (2022) and the results compared.*

<b>Date</b>	<b>P1 / P2</b>	<b>Slow method [MP/l]</b>	<b>Fast method [MP/l]</b>	<b>Deviation [%]</b>
06.06.2023	P1	19.4	21.987	11.8
06.06.2023	P2	12.933	14.227	9.1
07.06.2023	P1	9.053	15.52	41.7
07.06.2023	P2	11.64	16.813	30.8
13.06.2023	P1	11.64	10.347	-12.5
13.06.2023	P2	10.347	21.987	52.9
15.06.2023	P1	7.76	7.76	0.0
20.06.2023	P1	3.88	10.347	62.5
20.06.2023	P2	5.173	9.053	42.9
22.06.2023	P1	9.053	46.56	80.6
22.06.2023	P2	7.76	19.4	60.0
27.06.2023	P1	1.293	6.467	80.0
27.06.2023	P2	2.587	10.347	75.0
29.06.2023	P1	7.76	5.17	-50.0
29.06.2023	P2	12.93	9.05	-42.9
04.07.2023	P1	2.59	2.59	0.0
04.07.2023	P2	2.59	3.88	33.3
05.07.2023	P1	3.88	5.17	25.0
11.07.2023	P1	1.29	12.93	90.0
11.07.2023	P2	6.47	6.47	0.0
13.07.2023	P1	43.97	71.13	38.2
13.07.2023	P2	33.63	50.44	33.3