Microplastics in Drinking Water: Development and Automation of Raman Microspectroscopy

Measuring Microplastics Workshop

April 4, 2019
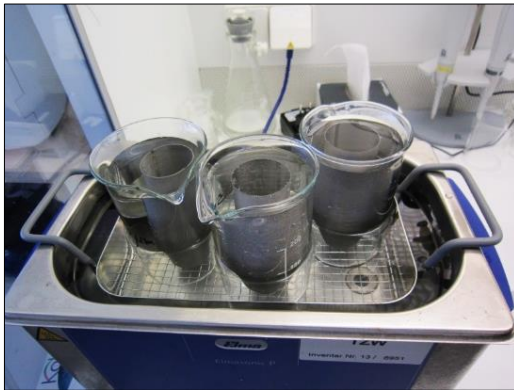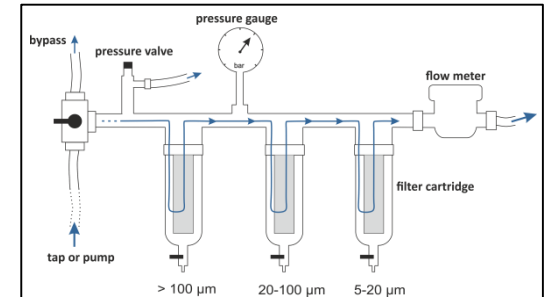
M. Pittroff, Y. K. Müller, C. S. Witzig, N. Zumbülte
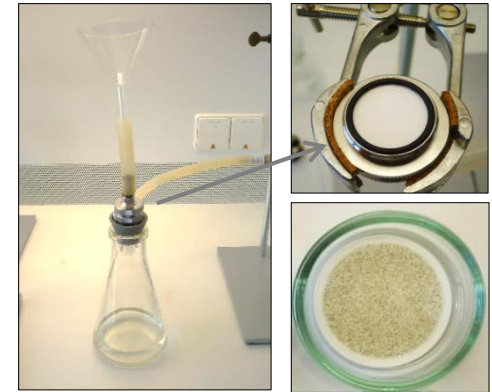
Workflow – Drinking Water

Sampling - filter cascade
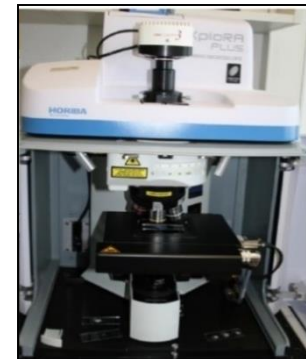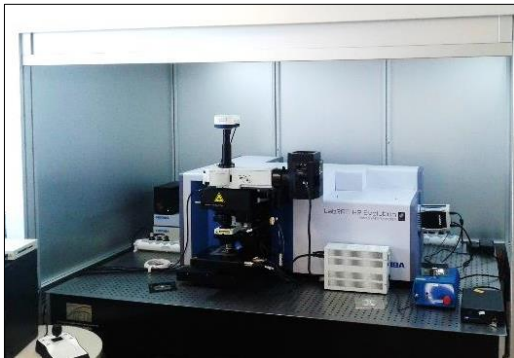→ 100 µm, 20 µm und 5 µm
(Volume up to 14 m³)

Sample preparation
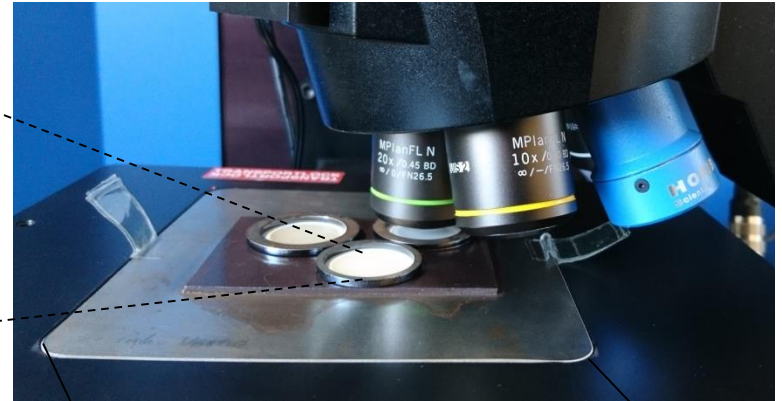- Ultrasonic extraction
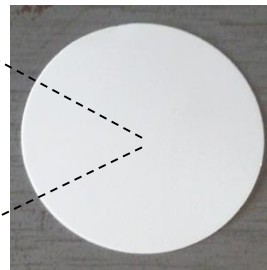- Vacuum filtration over PTFE filter

Detection/Identification
- Raman Microspectroscopy
- 20 – 50% of filter surface

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Sample Preparation – Filtration + Sample Stage

PTFE filter membrane
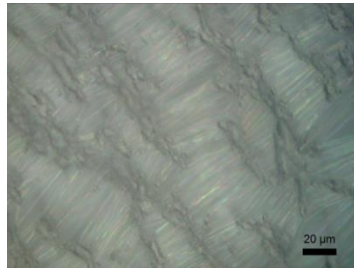
→ **1-2 µm pore size (25 mm diameter)**

→ **flat surface and good handling**

  (e.g. robustness, high chemical resistance)

Raman background

→ **no C-H bands (2900-3200 cm^{-1}) interference**

Selfmade sample stage

→ **Fixation under the microscope**

  (magnetic plate + steel ring)

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Horiba – Raman Systems

LabRAM HR Evolution
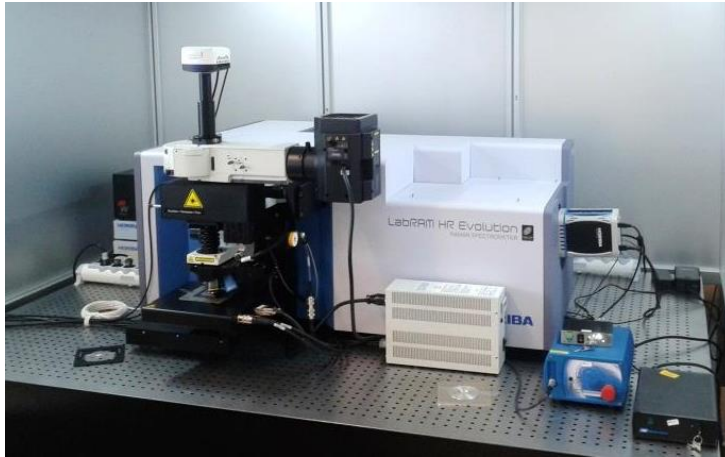
XploRA PLUS
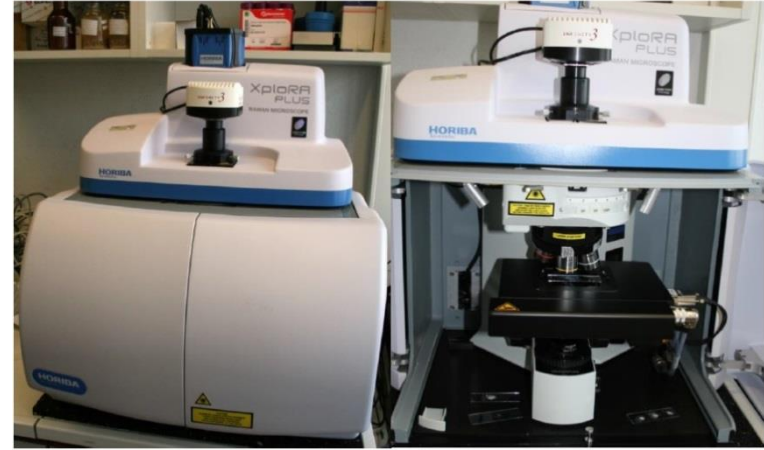
- **Detector:** EM-CCD detector
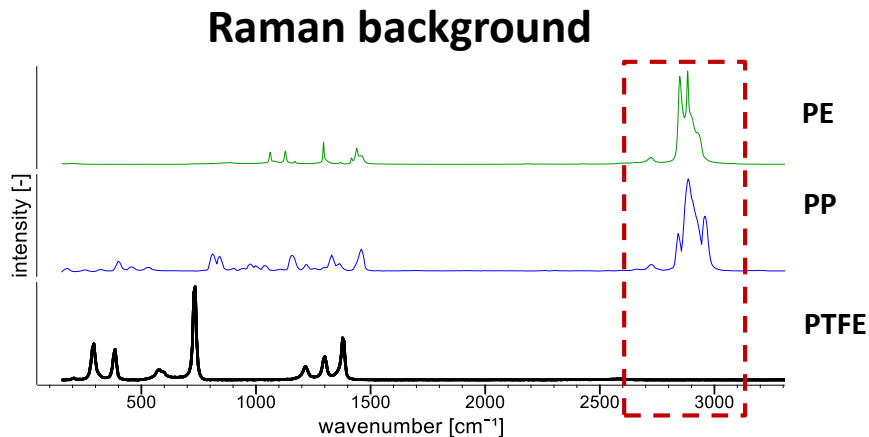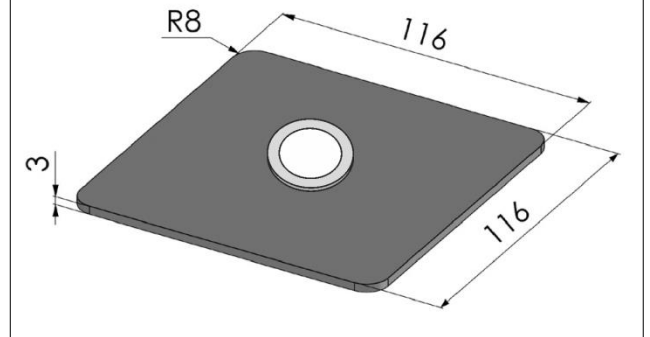
- **Objectives:** x5, x10, x20, x50, x100 (fully-motorized lens turret)

- **Resolution:** ≥ 1.4 cm^{-1} (spectral gratings: 300, 600, 1200, 1800 gr/mm)

- **Laser:** 532 nm (100mW), 633 nm (17mW), 785 nm (100mW)

- **Motorized xyz stage:** SWIFT XS Imaging (steps 0.5 μm; 1 ms) + DuoScan mode

- **Features:** 3D topography , Methods scripts, ParticleFinder (with Auto Focus)

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Microscope – Adaptable Imaging

- **Illumination modes**
- Mode is depending on measurement task (e.g. particles and filter substrate type)
- Best contrast between filter membrane and particles

reflection / transmission
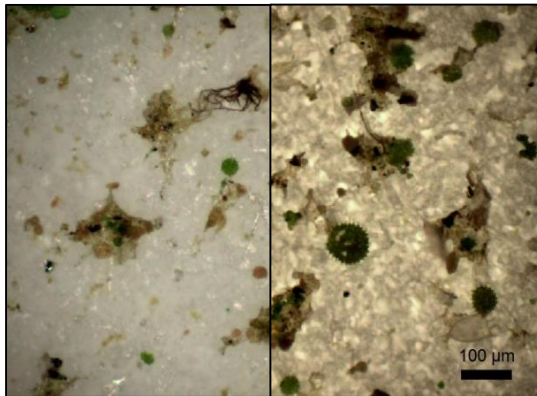
polarized light
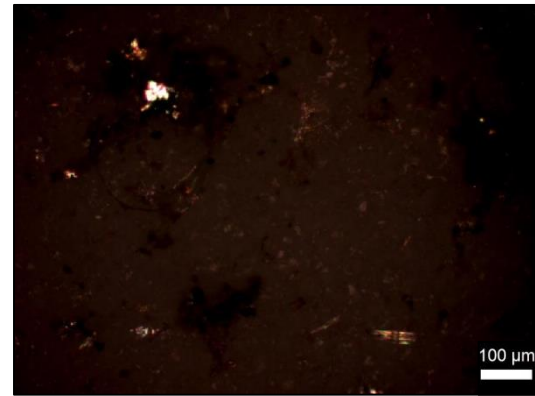
dark-field
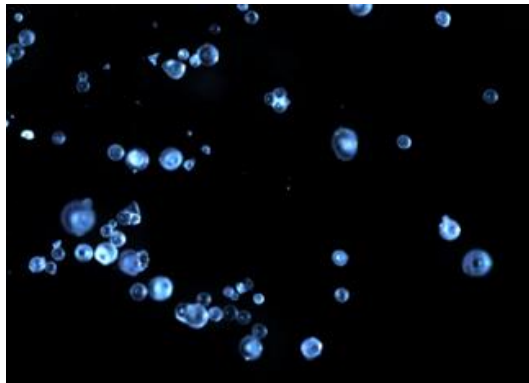
epi-fluorescence
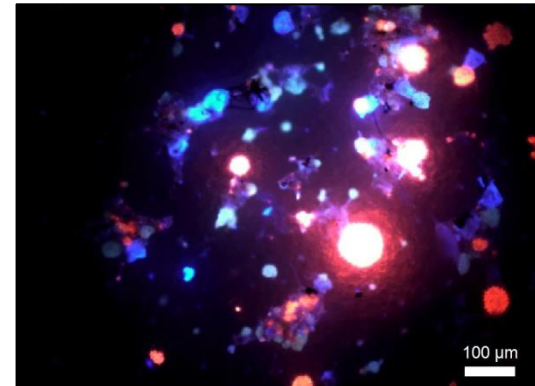
Analytic Workflow – Raman-Microspectroscopy

Particle Finder

Particle selection

Statistical analysis

> 5 µm

Raman acquisition

Data analysis

point-by-point mapping

Raman Imaging

Database search

< 5 µm

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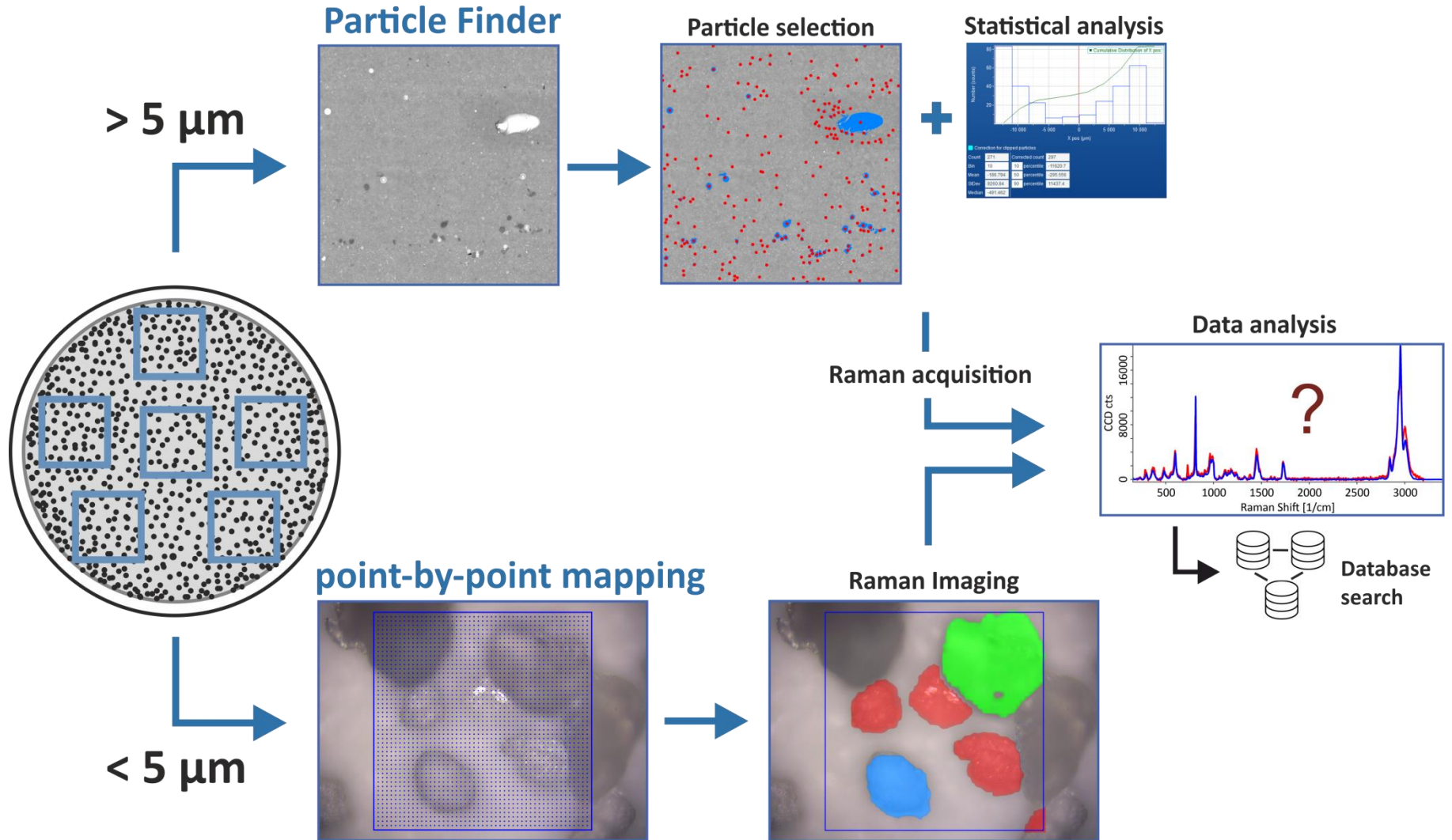
„ParticleFinder" Mosaic – In Detail

I. Particle identification

Mosaic (montaged) video Image

One of six section

single video images

montage of a large sample area
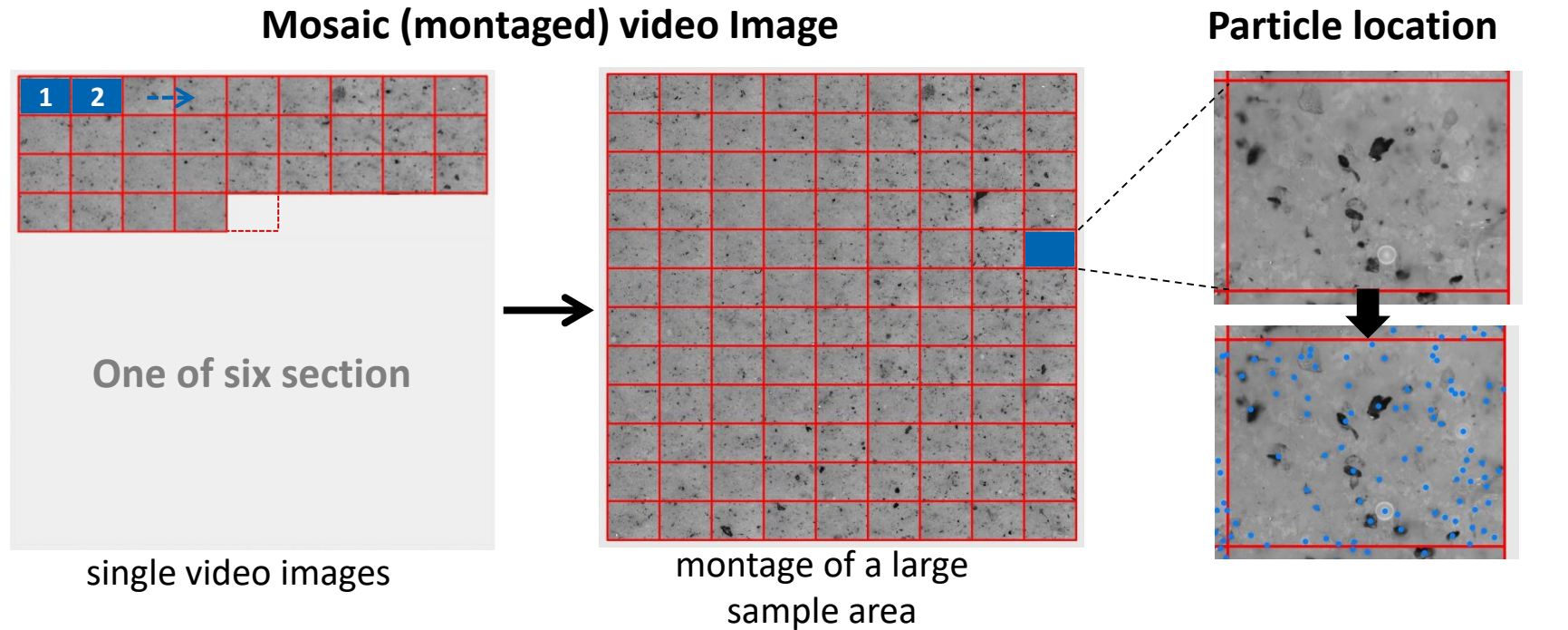
Particle location

- Individual **Auto Focus** for each image

- Black/white contrast

→ Raman Analysis is performed image by image

→ One Raman spectrum for each particle

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„ParticleFinder" Mosaic – Analytic Workflow

I. Particle identification

Mosaic (montaged) video Image

II. Particle size and shape

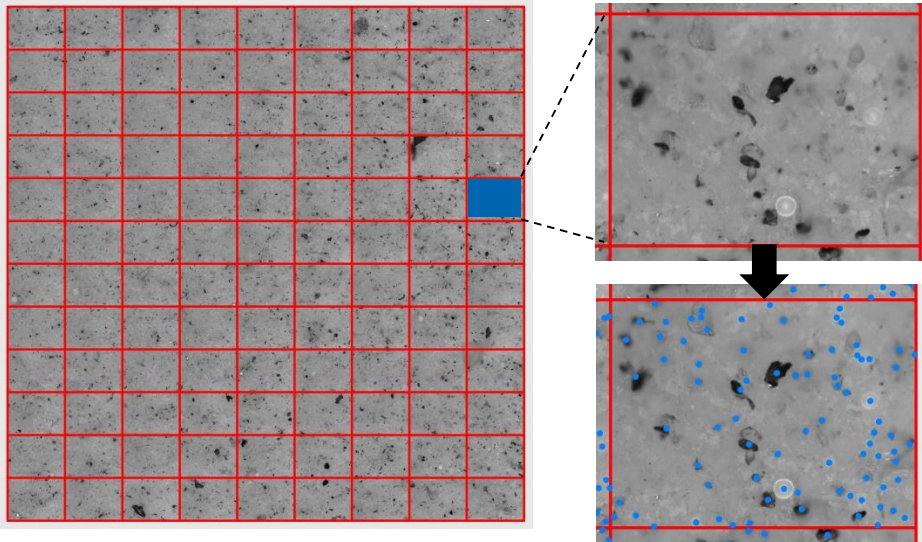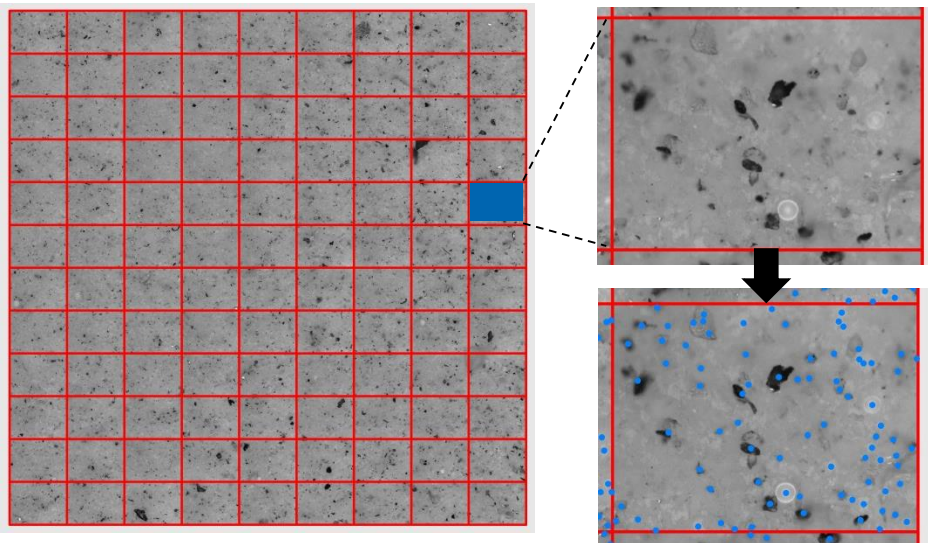| Include	Class	Index	X pos	Y pos	Area	Diameter	Perimeter	Major axis	Minor Axis	Ellipse ratio	Circularity	Brightness	Image	Raman
Use filtering			☐	☐	☐	☐	☐	☐	☐	☐	☐			
Filter limits(min)			-2049.0											
Filter limits(max)			2049.3											
☐		3431(3431)	1900.1	-431.7	53.3	8.2	102.0	15.0	6.9	0.46	0.25	166.3		
☐		3432(3432)	1792.9	-409.4	165.4	14.5	56.3	17.1	12.7	0.74	0.81	71.8		
☐		3433(3433)	1955.4	-409.2	58.2	8.6	42.4	9.8	8.2	0.83	0.64	113.5		
☐		3434(3434)	2001.9	-406.5	29.4	6.1	22.5	10.6	6.4	0.60	0.85	102.3		
☐		3435(3435)	1833.7	-393.1	596.0	27.5	262.1	33.6	29.1	0.87	0.33	179.1		
☐		3436(3436)	1722.2	-398.5	90.8	10.8	40.8	12.0	9.1	0.76	0.83	86.7		
☐		3437(3437)	1980.3	-395.5	35.5	6.7	38.5	12.0	0.0	0.00	0.55	171.7		

Table of results

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„ParticleFinder" Mosaic – Analytic Workflow

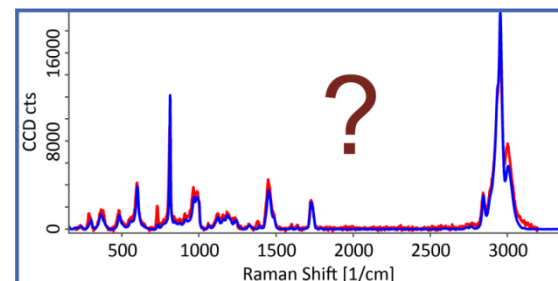## I. Particle identification

Mosaic (montaged) video Image

III. Chemical identification

Database search (semi-automated)

II. Particle size and shape

Include	Class	Index	X pos	Y pos	Area	Diameter	Perimeter	Major axis	Minor Axis	Ellipse ratio	Circularity	Brightness	Image	Raman
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Table of results

IV. Data extrapolation

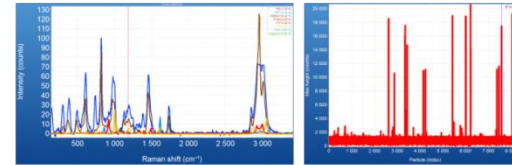**to all particles on the filter**

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„Raman Imaging" Mapping – Analytic Workflow

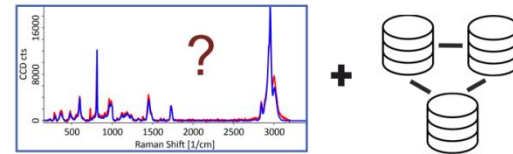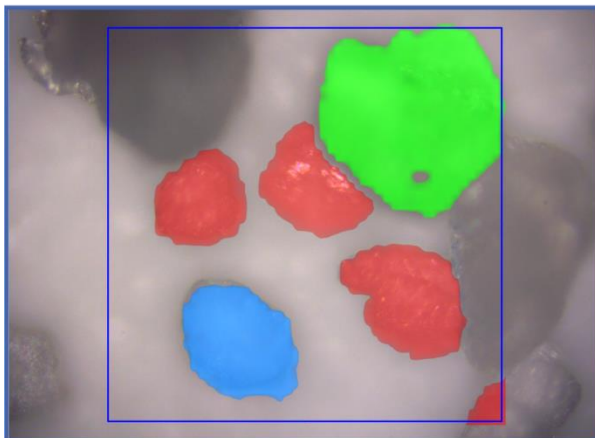## I. Point-by-point mapping
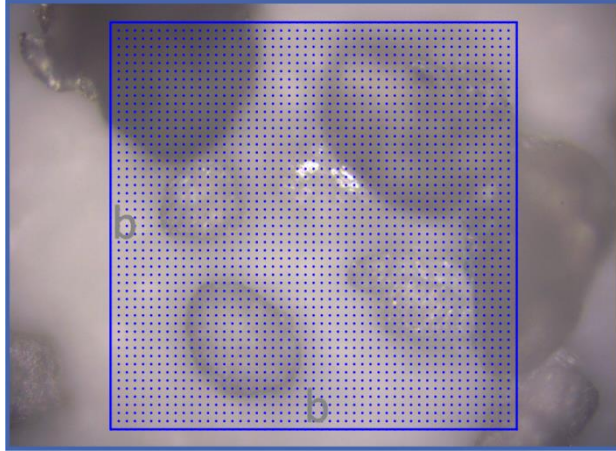
II. Chemical identification

CLS fitting

Database search
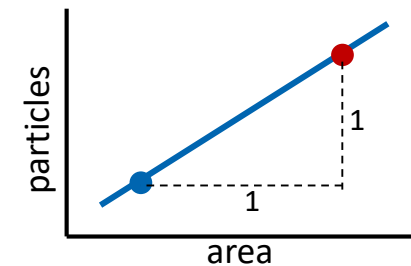

III. Particle Identification – Raman Imaging

PE
PVC
PS

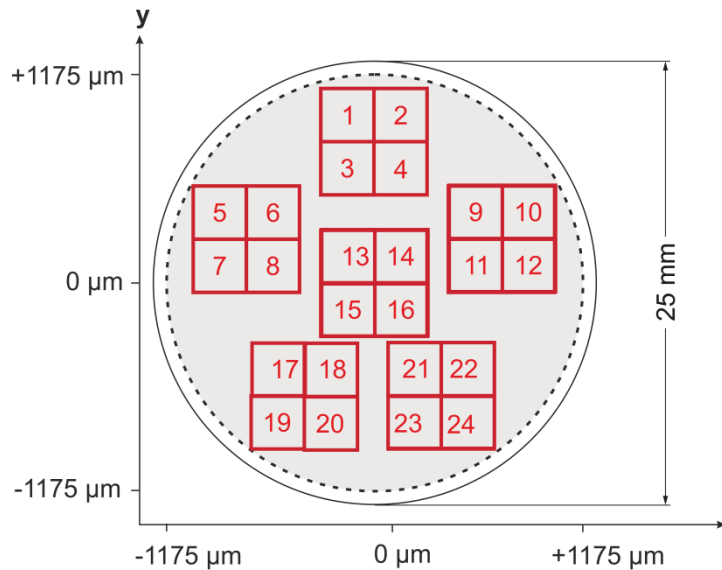## IV. Data extrapolation

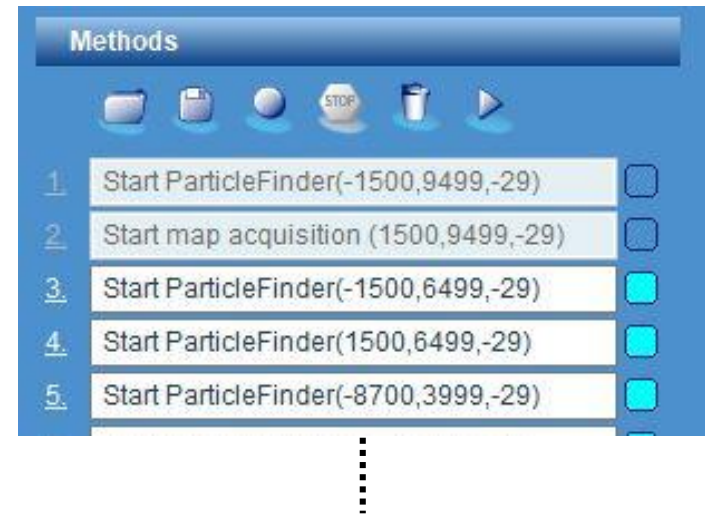**to all particles on the filter**

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In Practice? – Complex Procedure

- **Working with „Methods"**

Subdivision of sections
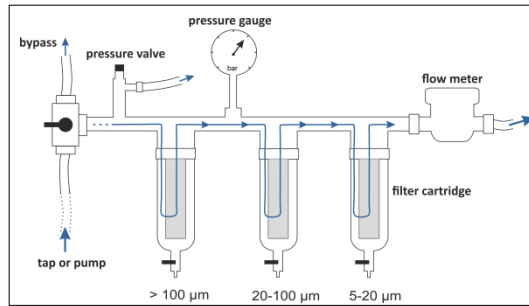

Customized „Methods" function

- 24 sub-sections of 3000 x 3000 µm

  (instead of 6 sections of 6000 x 6000 µm)

  = 24 separate ParticleFinder measurements

  with individual XYZ-coords

- Record a sequence of measurements

  and then replay the sequence

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In Practice? – Complex Procedure

- **Size fractionated sample**

One sample = three sub-samples (3 analysis)

1ˢᵗ sub-sample
2ⁿᵈ sub-sample
3ʳᵈ sub-sample
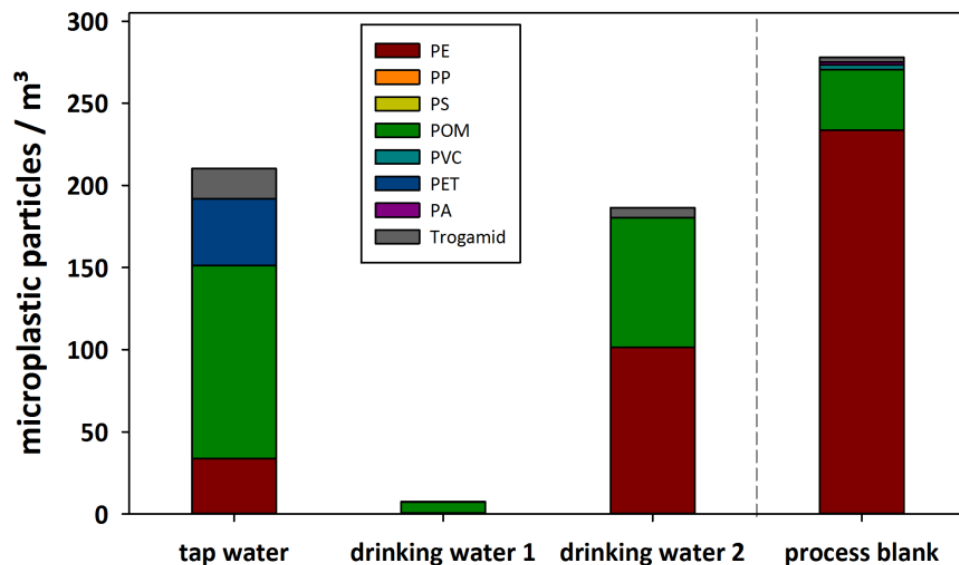
- **Time-consuming analysis** (e.g. 50% filter area)

Particle size:	< 5 µm	5 – 20 µm	> 20 µm
time / filter	> 1 week	4 to 8 days	1 to 4 days
particles / section	> 30,000	10,000 to 30,000	5,000 to 15,000

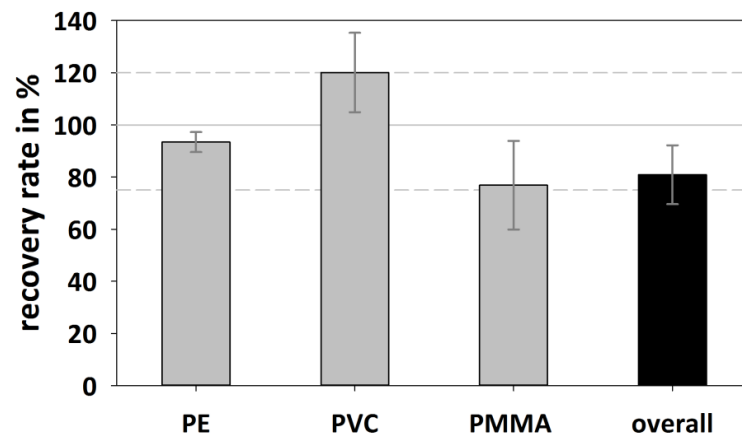→ **Measuring of the whole filter membrane is not feasible**

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Summary – Microplastic Analysis in **Drinking Water**
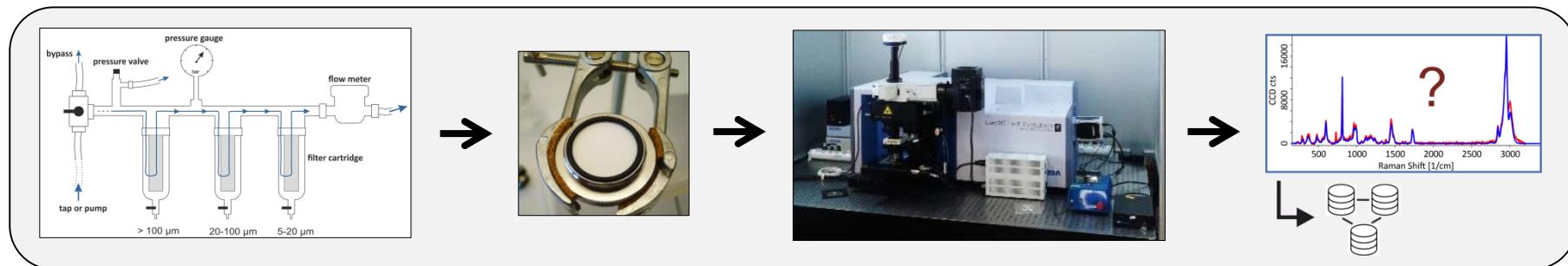
- **First results: Microplastics < Blank**

- **Validation of the method**

→ **Overall recovery of 81%**

→ **Microplastic analysis is established and validated**

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Involved colleagues of the
spectroscopy working group:

Yanina Müller

Cordula Witzig

Peter Habermehl

Dr. Nicole Zumbülte

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