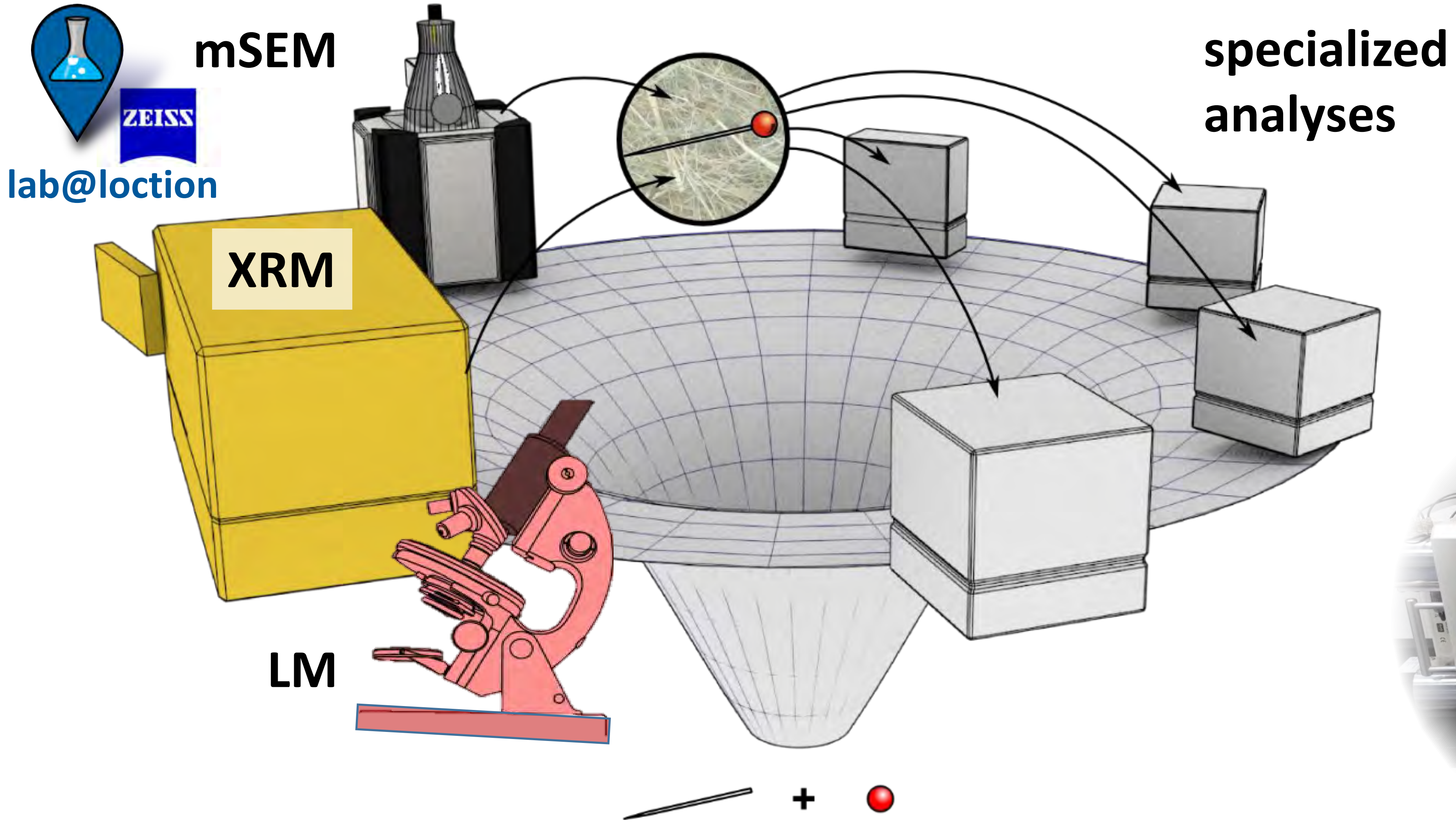
Raman spectroscopy in correlative & context workflows of microscopy & spectroscopy

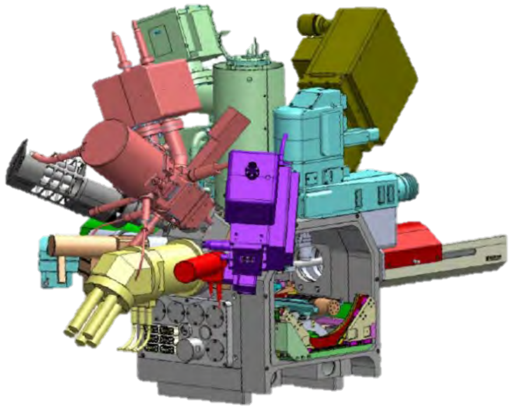
Silke Christiansen

HZB Helmholtz Zentrum Berlin
MPL
Fraunhofer IKTS

Correlative microscopy / spectroscopy

INAM

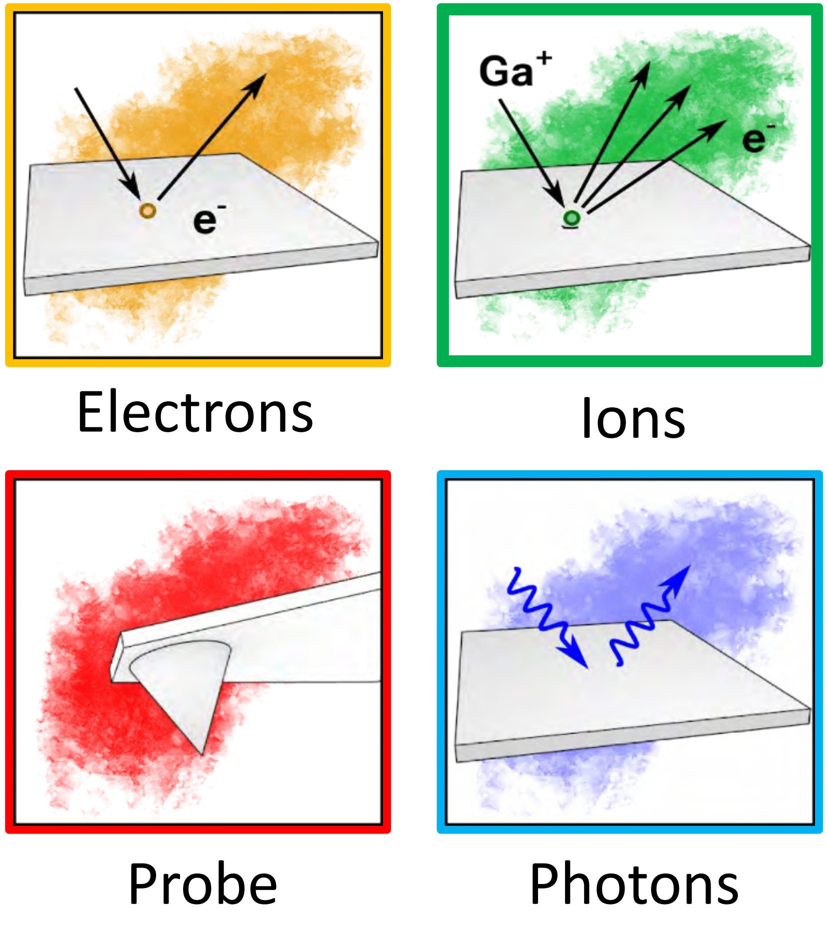


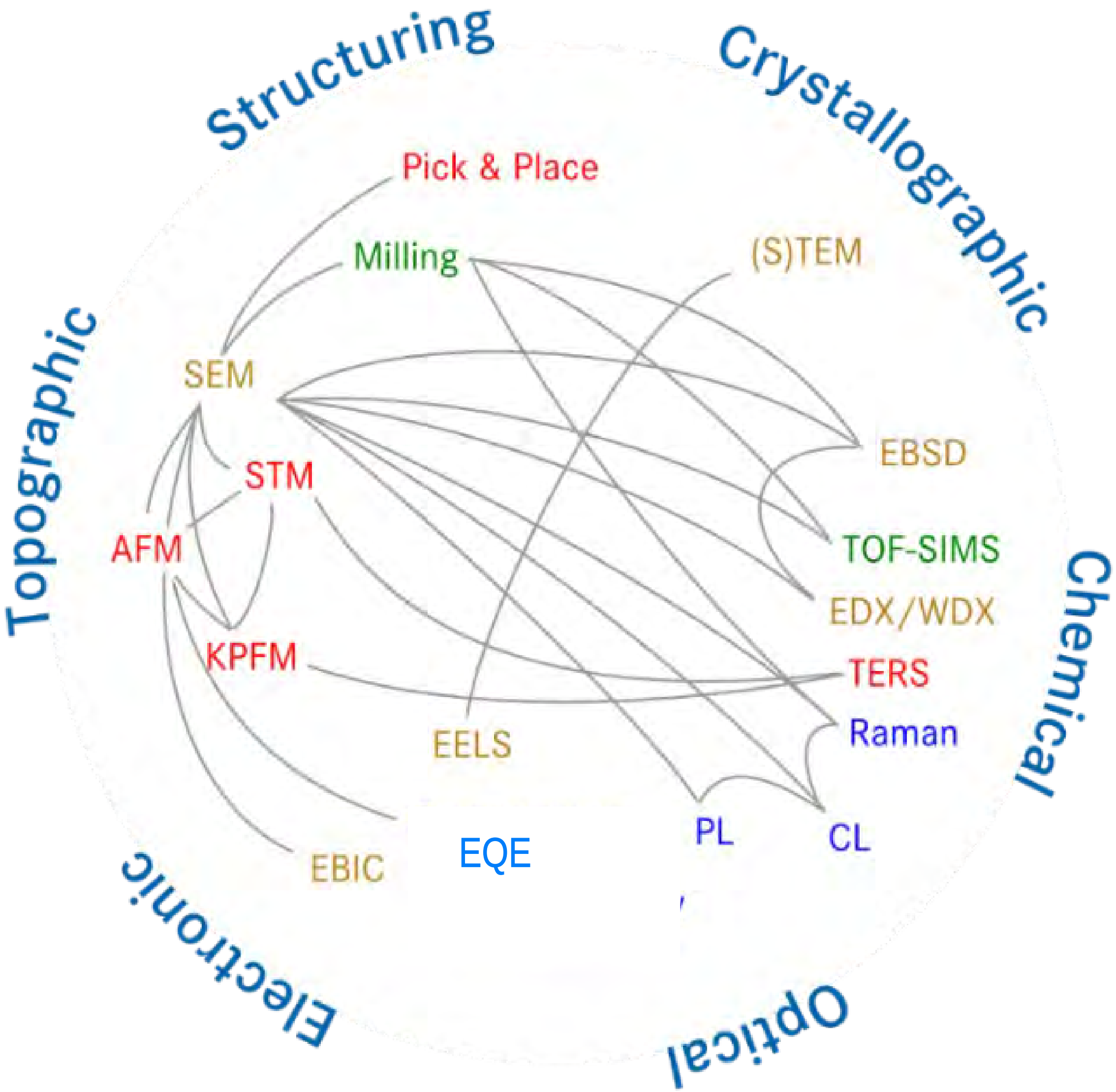


‚Big Data‘ & development of data mining strategies

Massive data reduction: data, properties and/or fit-parameter

Specialized analyses - various probes



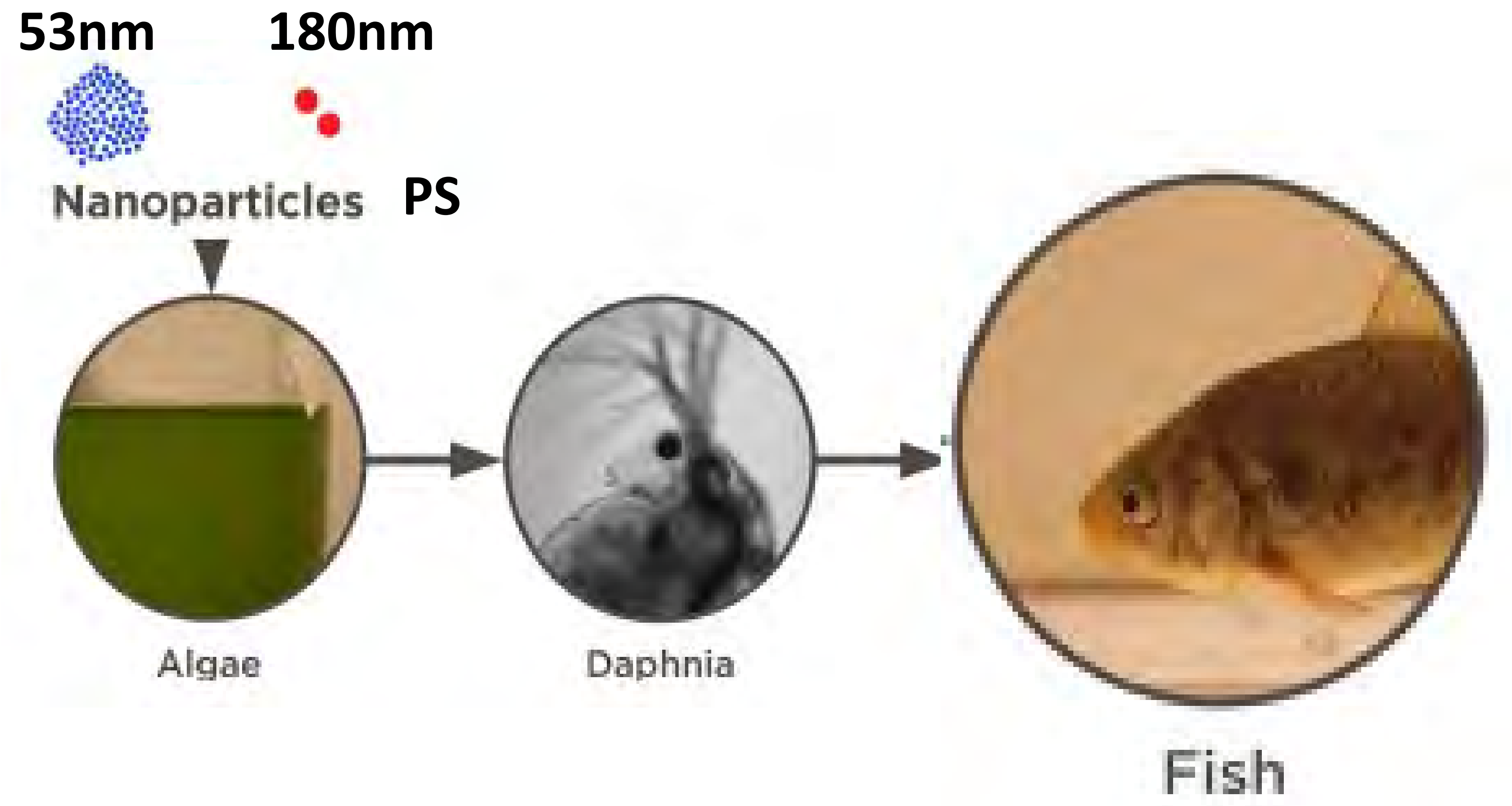


Outline

- **Standardization activities**
  - optimal filters for micro- and **nano-plastics**
  - **characterization workflows** – electron microscopy & spectroscopy
- **Wheathering of micro-/nano-plastics**
- **Example - mineral water samples**
  - Nanoplastics
  - Pigments
  - Additives

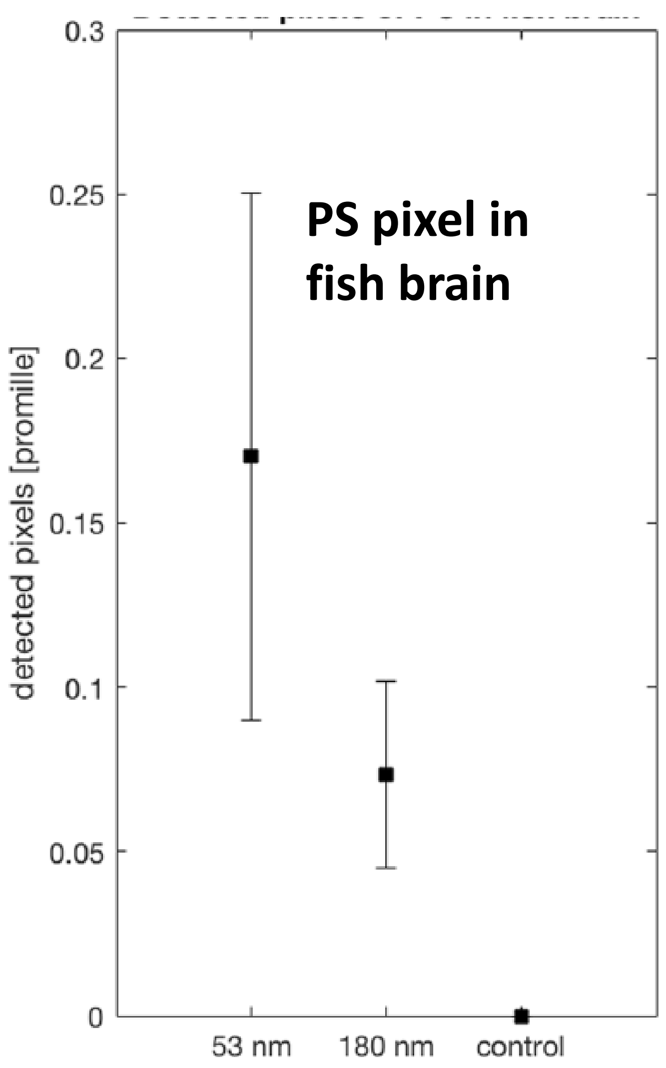
Alarming: nano-plastics crossed blood-brain barrier in fish

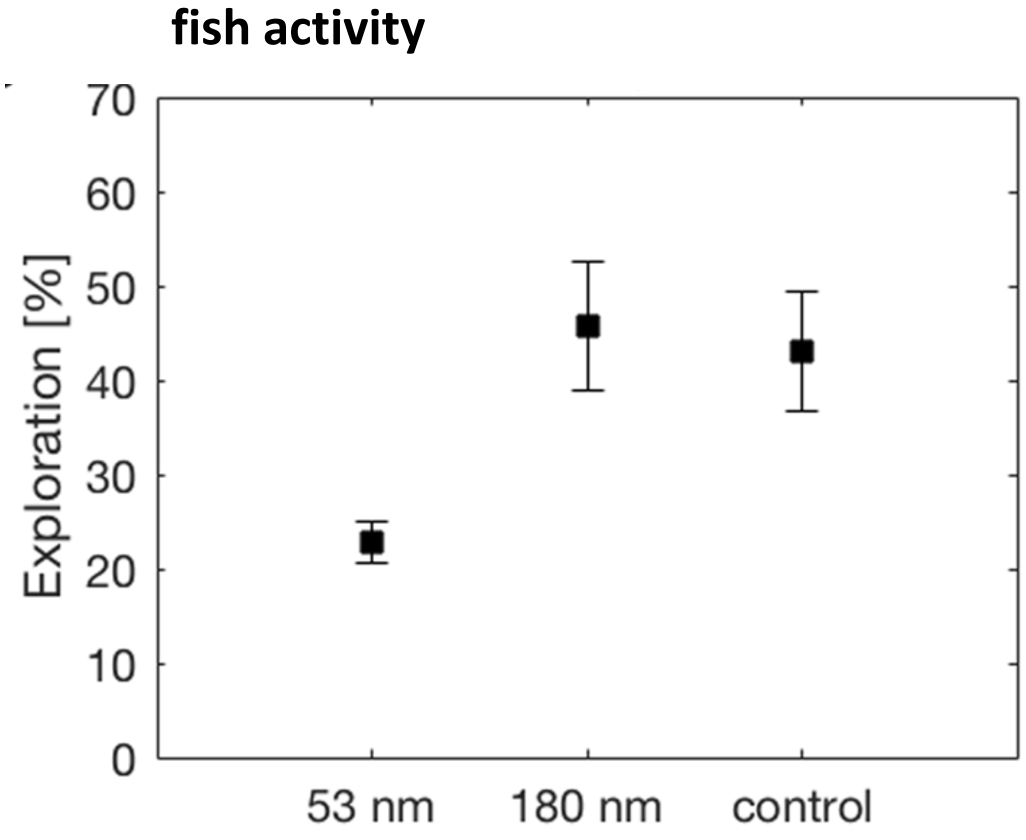
Food-chain



Alarming: nano-plastics crossed blood-brain barrier in fish

International Agency for **Research on Cancer (IARC)** since 2013:
fine dust **(particles <2.5µm)**
identified to be **carcinogenic**





K. Mattsson, E. V. Johnson, A. Malmendal, S. Linse, L.A. Hansson, T. Cedervall, Sci. Rep. 7, 11452 (2017)

Outline

- **Standardization activities**
    - optimal filters for micro- and **nano-plastics**
    - **characterization workflows** – correlative microscopy & spectroscopy
- **Wheathering of micro-/nano-plastics**
- **Example - mineral water samples**
    - Nanoplastics
    - Pigments
    - Additives

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

Number & size of micro-/nano-plastics in mineral water

	Microplastics/l								
	single use PET			reusable PET			glass		
	Oßmann et al. (2018)		Schymanski et al. 2018)	Oßmann et al. (2018)		Schymanski et al. (2018)	Oßmann et al. (2018)		Schymanski et al. (2018)
≤1.5 µm	1419±1614			2298±3048			1031±1773		
>1.5 µm to ≤5 µm	1184±1329			2365±2457			3860±1746		
>5 µm to ≤10 µm	45±64	45±64	14±14	143±226	226±307	118±88	967±1779	1401±2588	50±52
>10 µm	0±0			83±136			434±715		
total	2648±2857			4889±5432			6292±10521		

38 bottles: **D. Schymanski**, C. Goldbeck, H. U. Humpf, P. Fürst, Water Research 129 154-162 (2018)

32 bottles: **B. E. Oßmann**, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

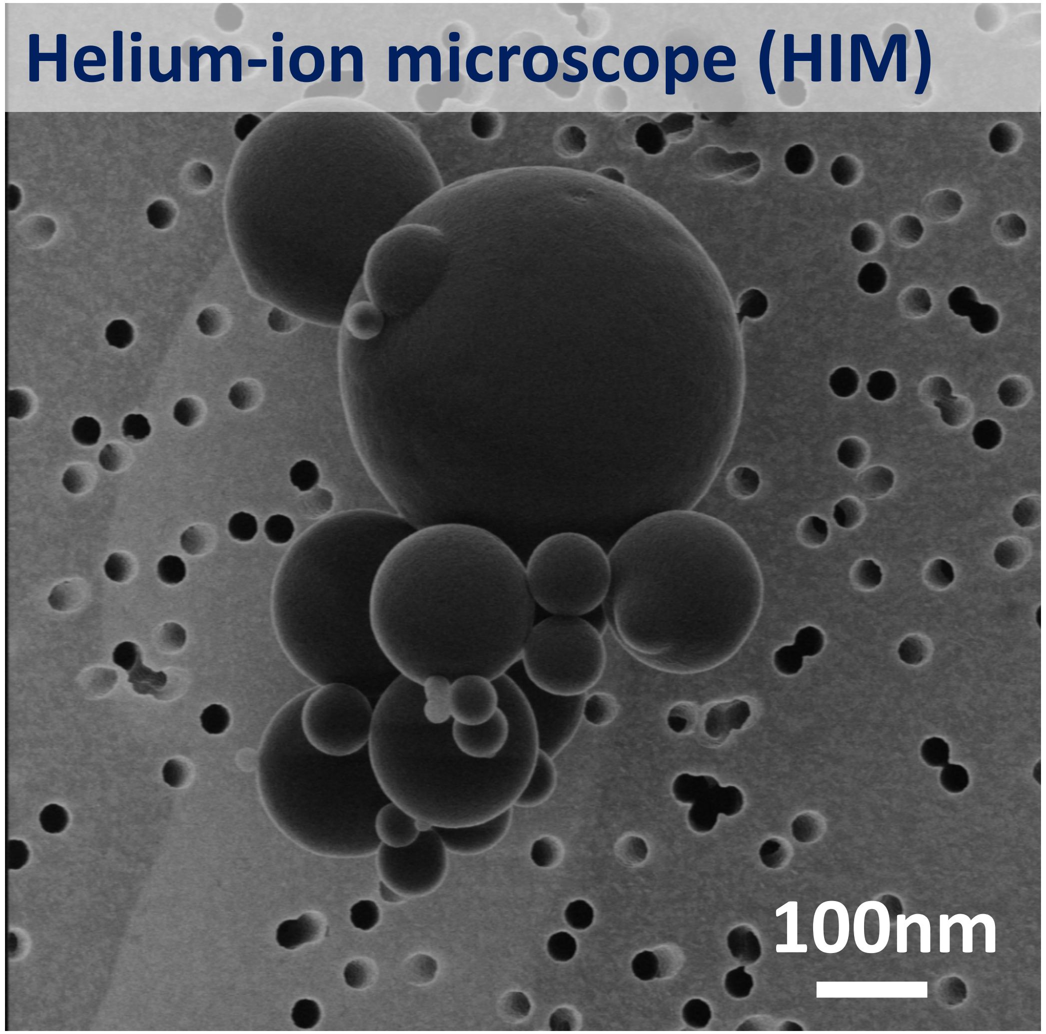
Outline

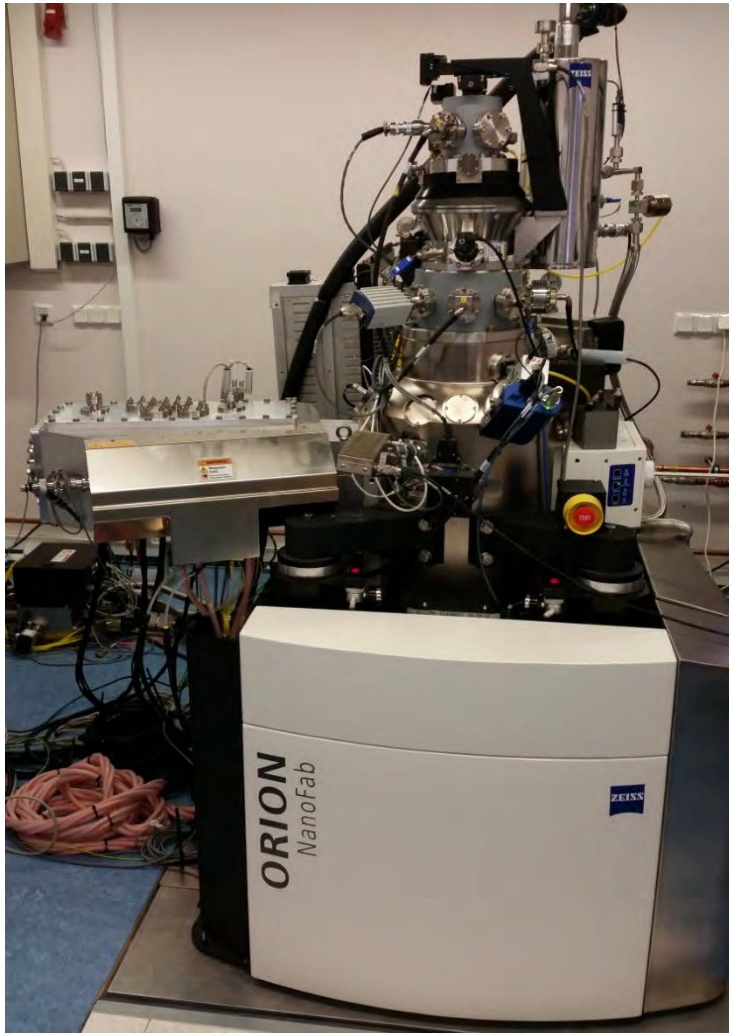
- **Standardization activities**
  - optimal filters for micro- and **nano-plastics**
  - **characterization workflows** – electron microscopy & spectroscopy
- **Wheathering of micro-/nano-plastics**
- **Example - mineral water samples**
  - **Nanoplastics**
  - **Pigments**
  - **Additives**

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

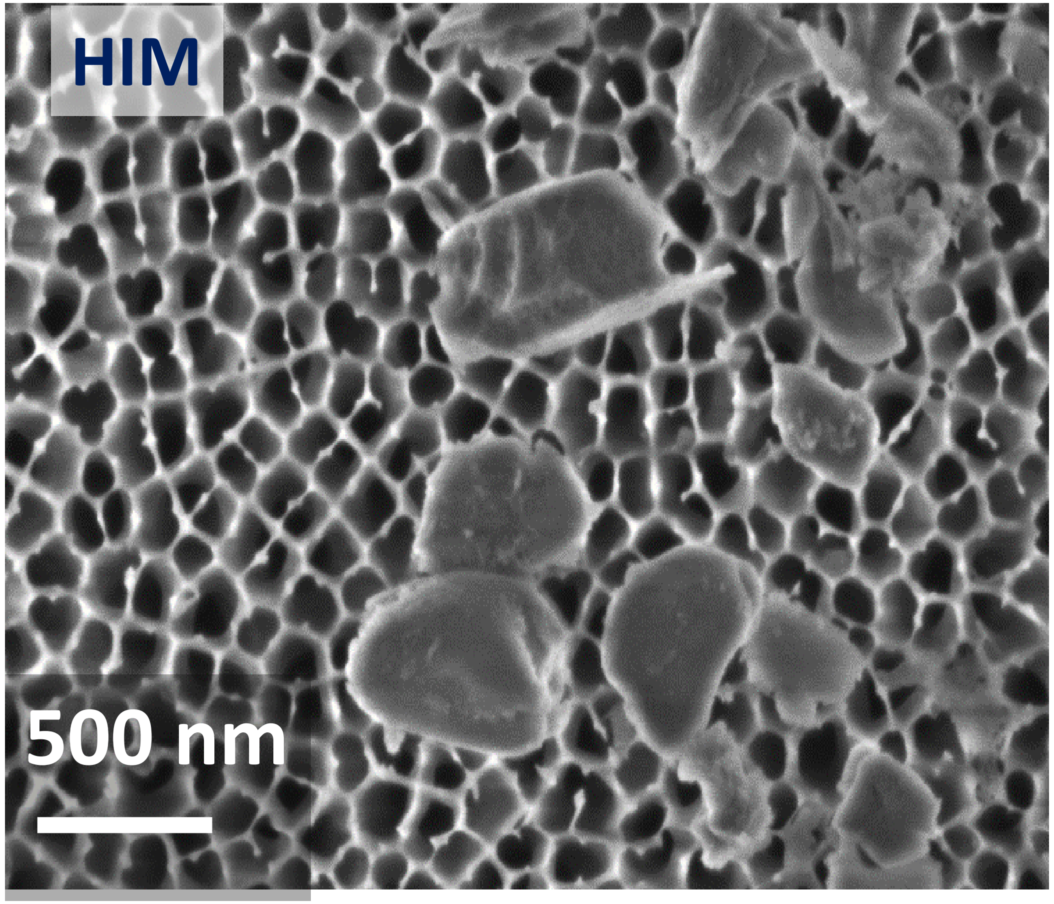
Nano-plastic particles on PC filter membrane

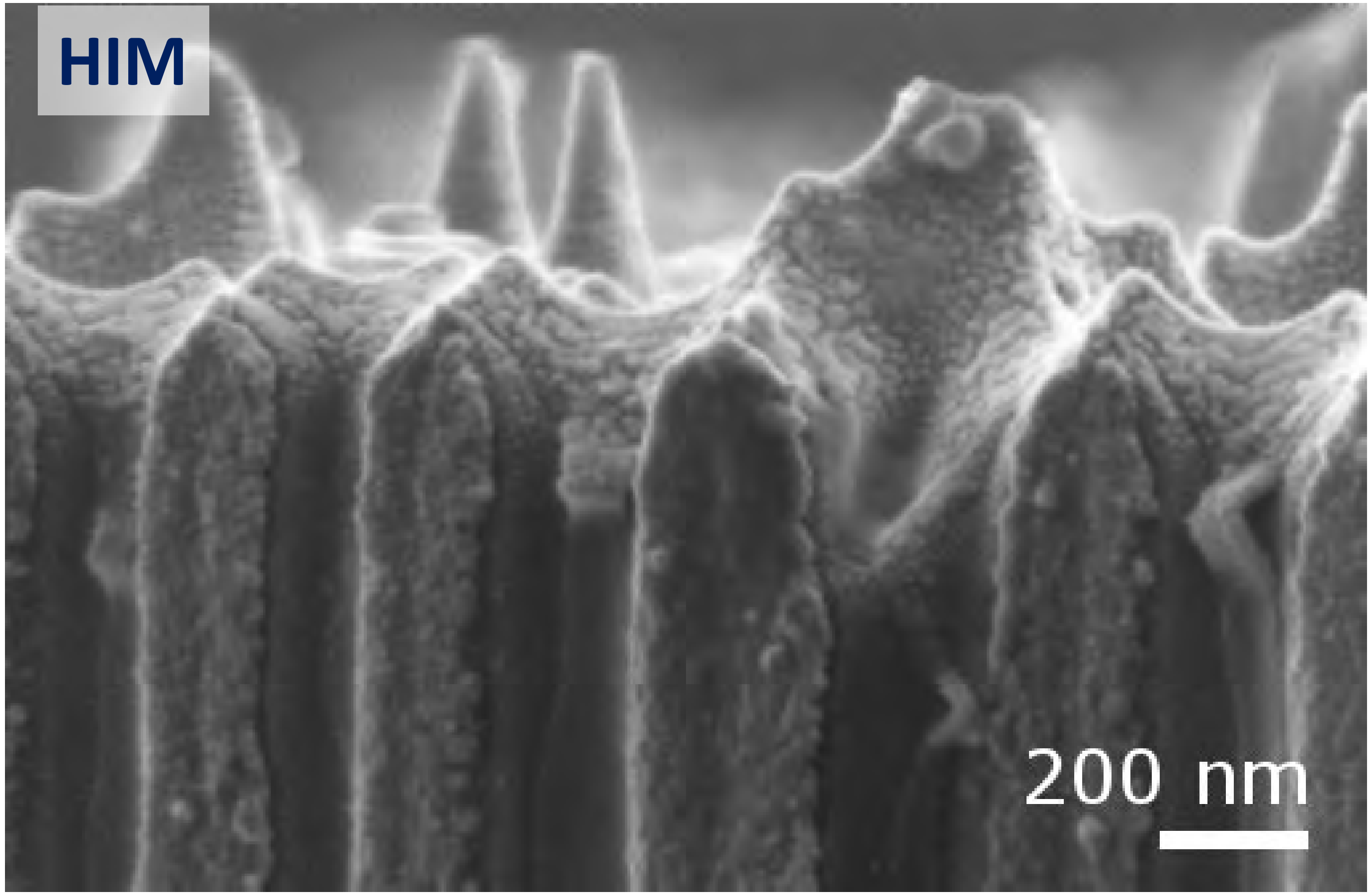




B. Oßmann, G. Sarau, L. Kling, S. Christiansen, unpublished

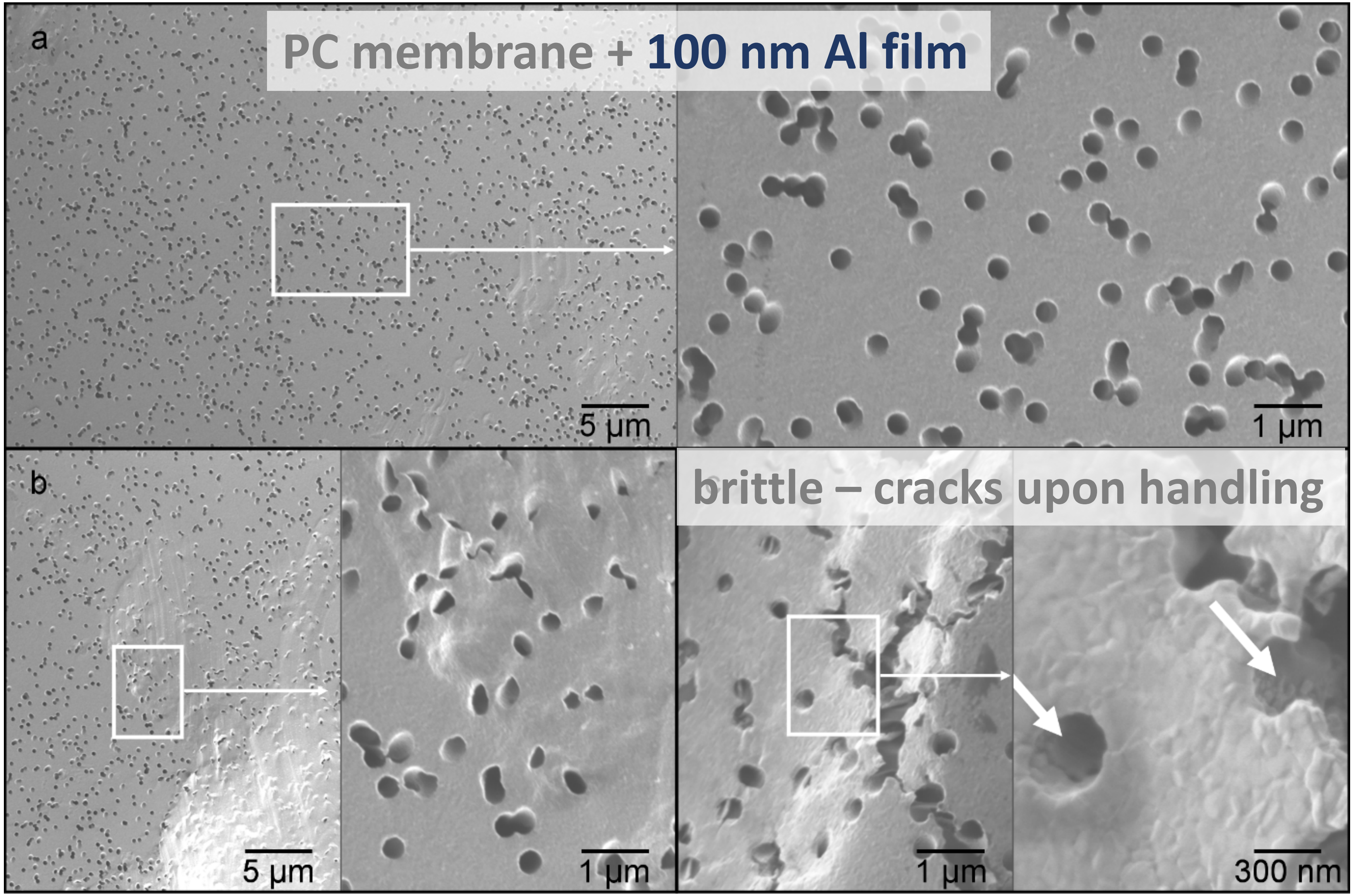
Nano-plastic particles on AlO_x filter membrane





Filter membrane – nano-holes

SEM



Filter membrane selection

Material of the membrane filter	Smooth and unstructured surface	Applicability of Particle Finder module		Raman background of the filter material		Maximum laser power for non-destructive Raman measurement of all standard particles (mW)	
		Illumination		Laser wavelength			
		Bright field	Dark field	532 nm	785 nm	532 nm	785 nm
Regenerated Cellulose	No	Not tested[a]					
Nitrocellulose	No						
Cellulose acetate	No						
Aluminium oxide	No						
Silver	No						
PC white	Yes	No	No	Intensive Raman spectrum		Not tested[a]	
PC black	Yes	No	Yes	Intensive Raman spectrum		Not tested[a]	
PC coated with gold	Yes	No	Yes	Weak fluorescence		~1.2	~5.3
PC white coated with titan	Yes	No	Yes	Burning		Not possible[b]	
PC white coated with nickel	Yes	No	Yes	No background	Burning	~1.2	Not possible[b]
PC white coated with aluminium	Yes	No	Yes	No background	Weak fluorescence	~3.2	~5.3

[a] Material was excluded from further tests because of prior results

[b] Non-destructive Raman measurement was not possible due to burning of particles

PC Polycarbonate

- Pore size < 1 or 0.5 µm
- Smooth and unstructured surface for good visibility of particles ≤ 1 µm
- High optical contrast for automatic particle detection
- No spectral interference from substrate (Raman, Fluorescence)
- No burning of particles with one or both lasers

B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

Filter membrane selection

Material of the membrane filter	Smooth and unstructured surface	Applicability of Particle Finder module		Raman background of the filter material		Maximum laser power for non-destructive Raman measurement of all standard particles (mW)	
		Illumination		Laser wavelength			
		Bright field	Dark field	532 nm	785 nm	532 nm	785 nm
Regenerated Cellulose	No	Not tested[a]					
Nitrocellulose	No						
Cellulose acetate	No						
Aluminium oxide	No						
Silver	No						
PC white	Yes	No	No	Intensive Raman spectrum		Not tested[a]	
PC black	Yes	No	Yes	Intensive Raman spectrum		Not tested[a]	
PC coated with gold	Yes	No	Yes	Weak fluorescence		~1.2	~5.3
PC white coated with titan	Yes	No	Yes	Burning		Not possible[b]	
PC white coated with nickel	Yes	No	Yes	No background	Burning	~1.2	Not possible[b]
PC white coated with aluminium	Yes	No	Yes	No background	Weak fluorescence	~3.2	~5.3

[a] Material was excluded from further tests because of prior results

[b] Non-destructive Raman measurement was not possible due to burning of particles

PC Polycarbonate

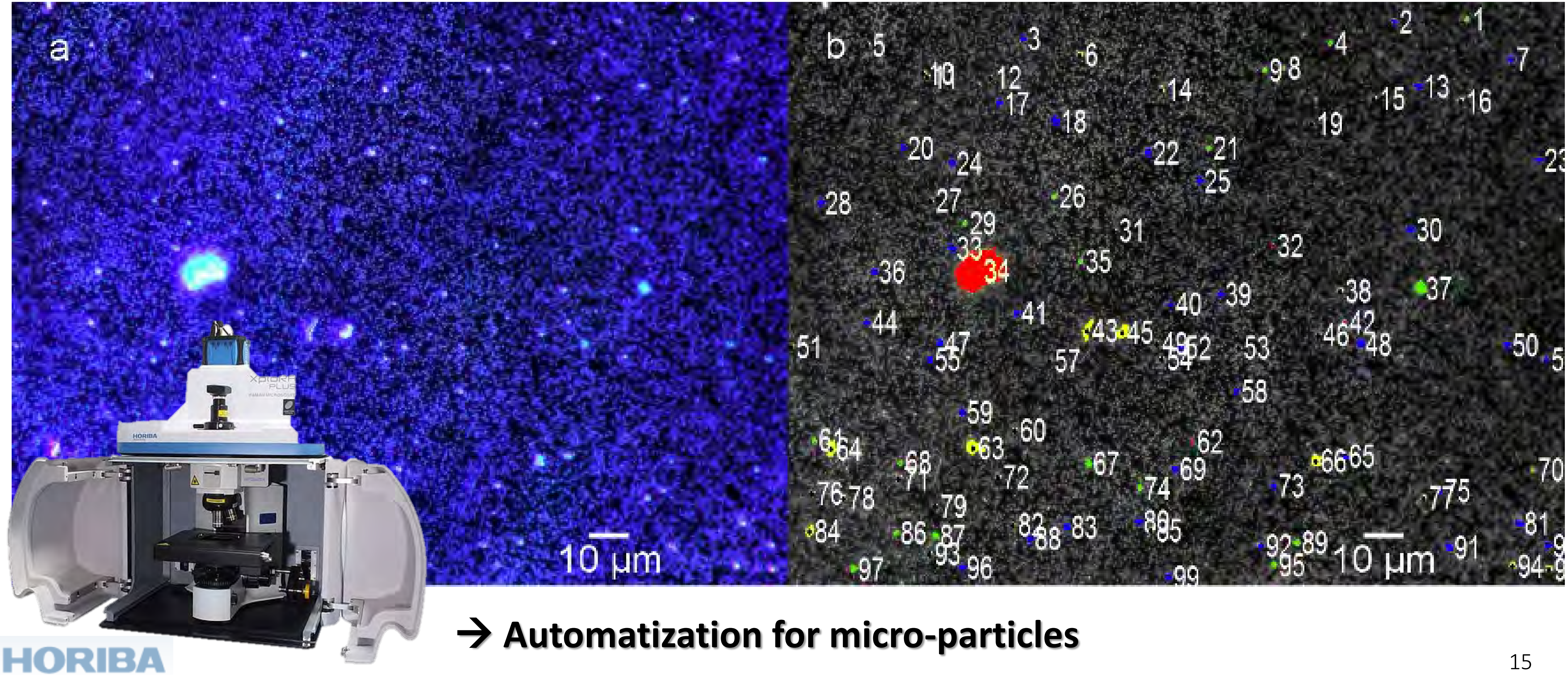
- Pore size < 1 µm
- Smooth and unstructured surface for good visibility of particles ≤ 1 µm
- High optical contrast for automatic particle detection
- No spectral interference from substrate (Raman, Fluorescence)
- No burning of particles with one or both lasers

B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

Optical microscopy – dark field

INAM

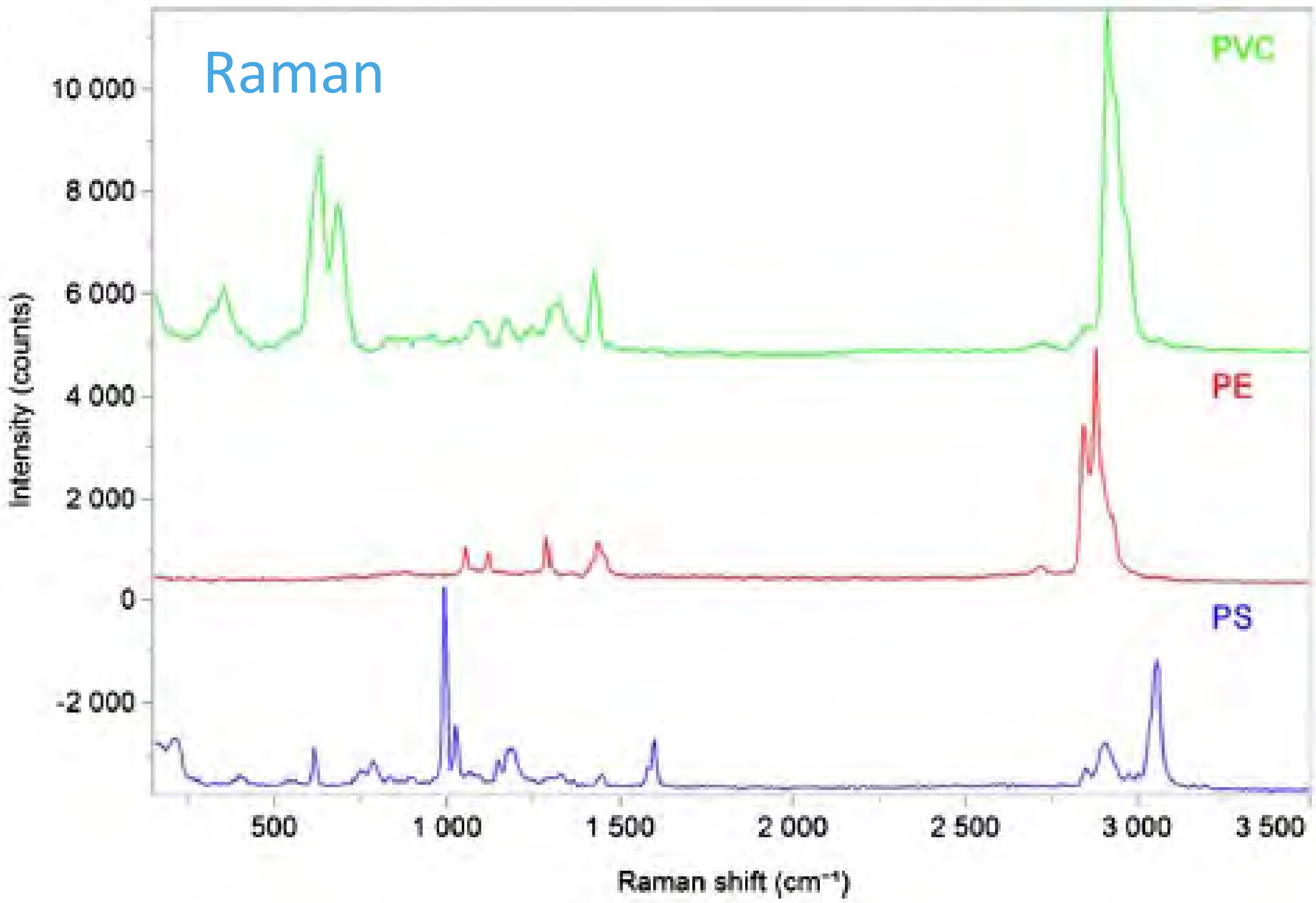
Dark field **Particle finder**



HORIBA Scientific

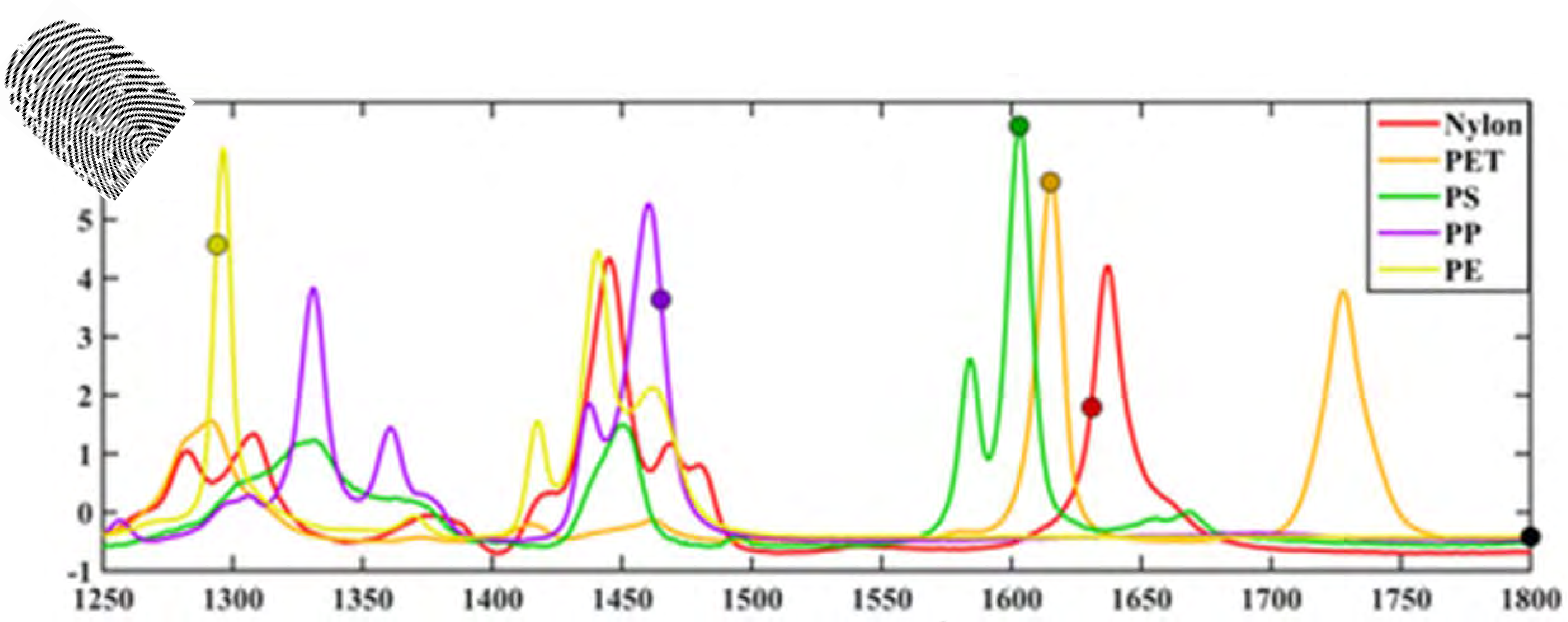
→ Automatization for micro-particles

Raman spectroscopy – molecular fingerprinting



Raman spectroscopy – plastics fingerprinting

INAM



C.F. Araujo et al., *Water Research* 142, 426 (2018)

Outline

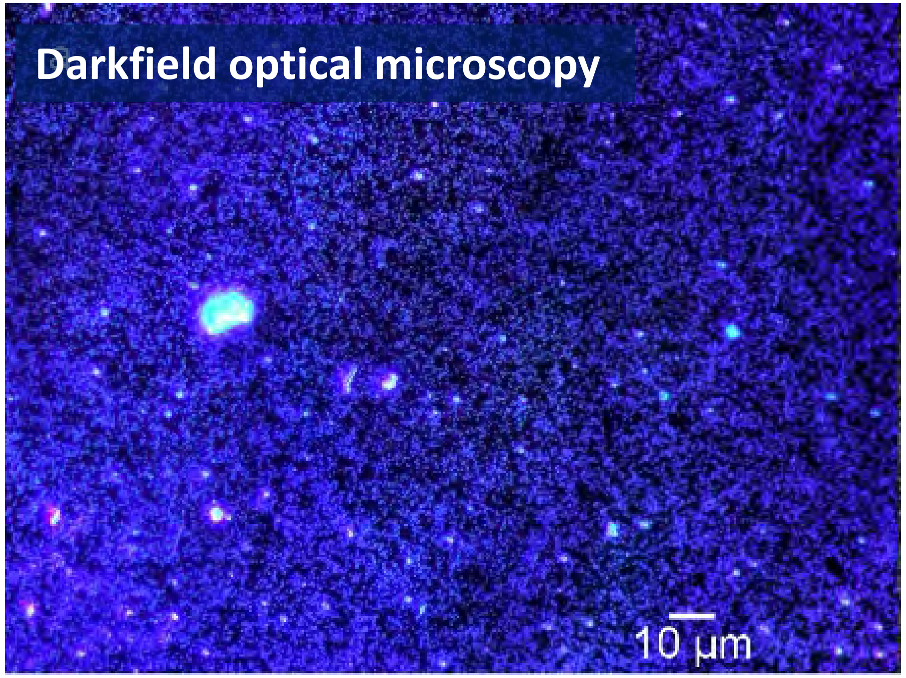
- **Standardization activities**
  - optimal filters for micro- and **nano-plastics**
  - **characterization workflows** – electron microscopy & spectroscopy
- **Wheathering of micro-/nano-plastics**
- **Example - mineral water samples**
  - **Nanoplastics**
  - **Pigments**
  - **Additives**

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

Outline

- **Standardization activities**
  - optimal filters for micro- and **nano-plastics**
  - **characterization workflows** – electron microscopy & spectroscopy



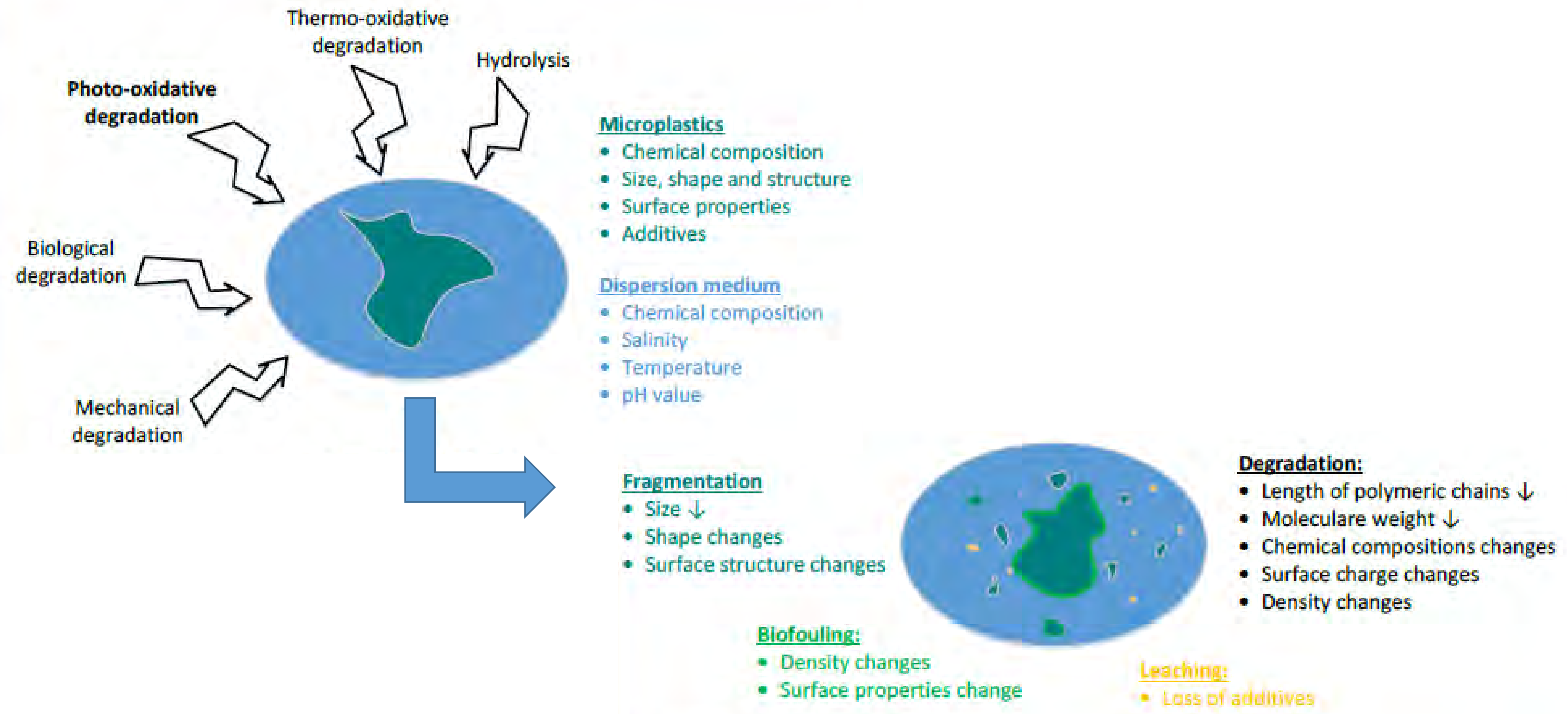
Outline

- **Standardization activities**
  - optimal filters for micro- and **nano-plastics**
  - **characterization workflows** – correlative microscopy & spectroscopy
- **Wheathering of micro-/nano-plastics**
- **Example - mineral water samples**
  - Nanoplastics
  - Pigments
  - Additives

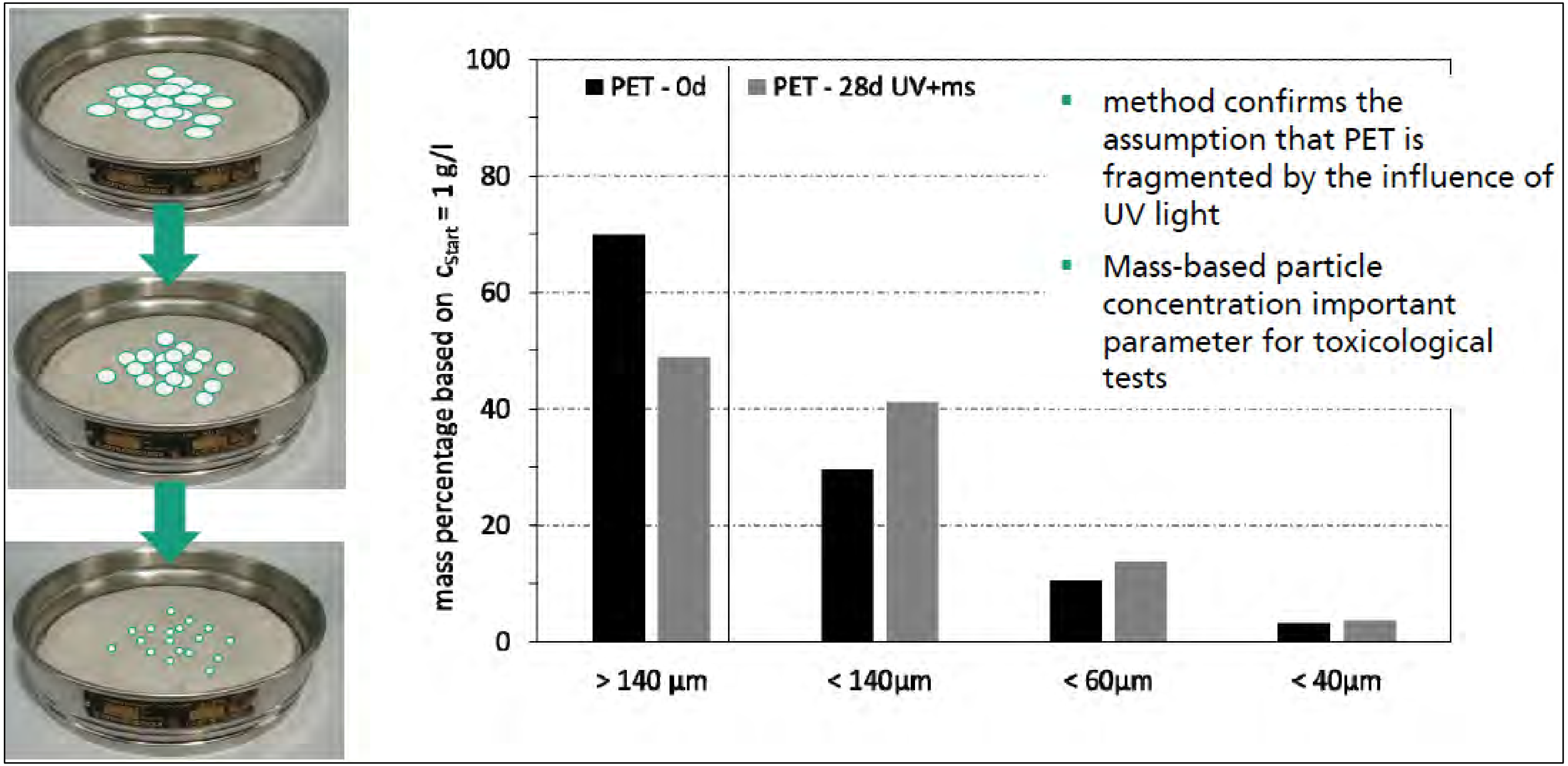
B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

Wheathering of microplastic - degradation



Effect of UV light exposure



- method confirms the assumption that PET is fragmented by the influence of UV light
- Mass-based particle concentration important parameter for toxicological tests

K. Oelschlägel, J. Pfeiffer, A. Potthoff, Fraunhofer Institute for Ceramic Technologies and Systems IKTS, Dresden, Germany, unpublished

Effect of UV light exposure

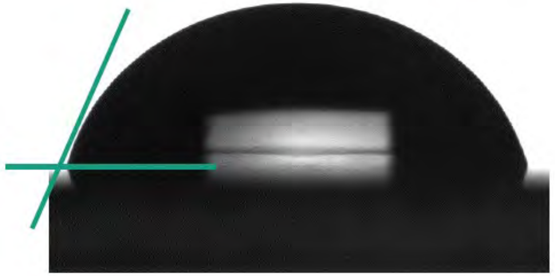
Fraunhofer IKTS

Hydrophilicity ↑

Non-weathered PET slide

Contact angle: 73,8° ± 1,2°

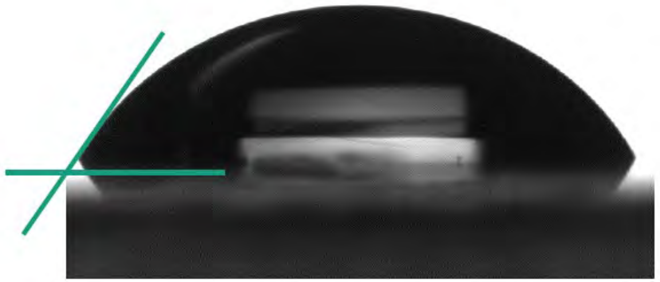
Zeta potential: -32 mV ± 2 mV



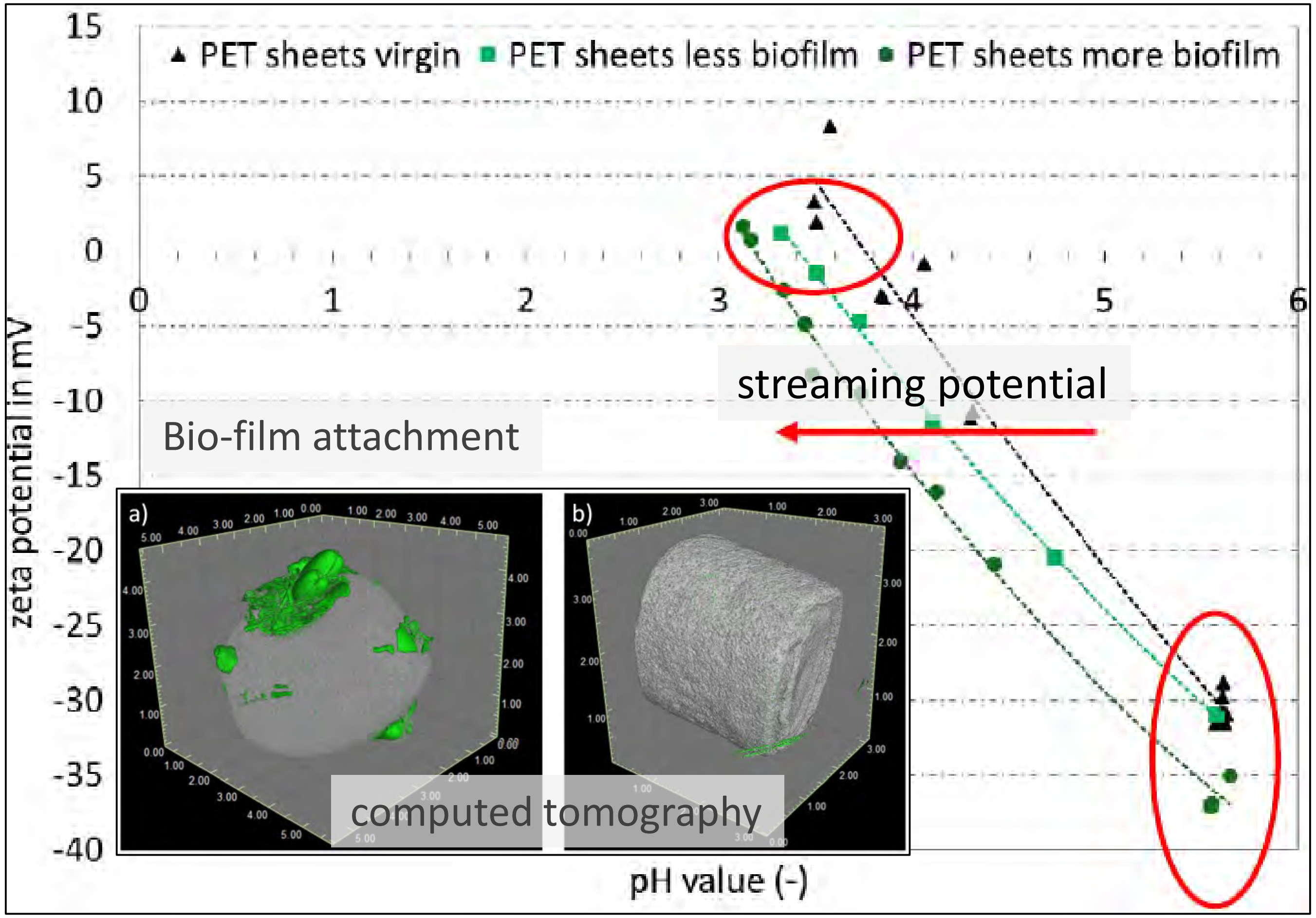
PET slide weathered with UV radiation

Contact angle: 61,5° ± 1,0°

Zeta potential: -38 mV ± 4 mV



Zeta potential ↓ Bio-film ↑



K. Oelschlägel, J. Pfeiffer, A. Potthoff, Fraunhofer Institute for Ceramic Technologies and Systems IKTS, Dresden, Germany, unpublished

Correlative workflow in Zen Connect

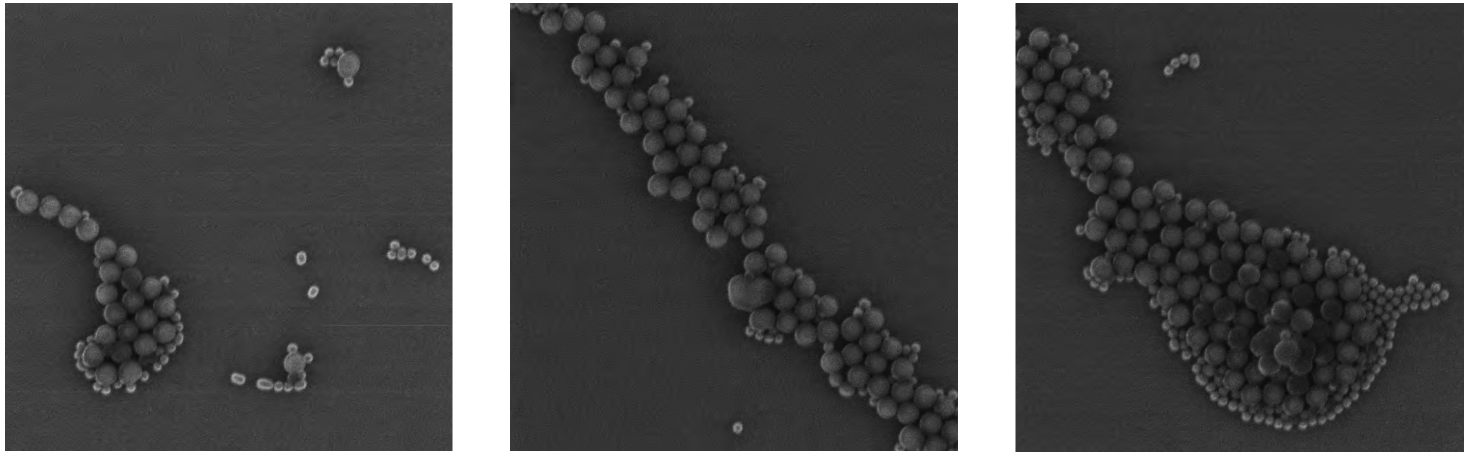
Zen connect:

- Multi-modal data overlay
- organize and overlay data from any source
- Rise microscope: Witec Raman in a Zeiss Sigma SEM

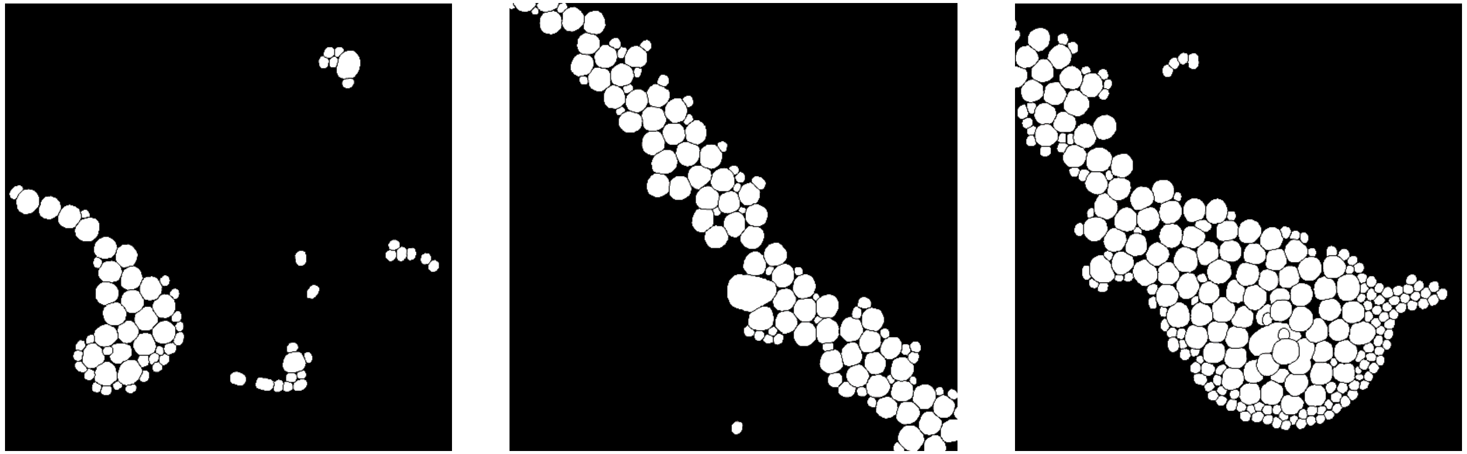
https://www.zeiss.com/microscopy/int/products/microscope-software/zen-connect-image-overlay-and-correlative-microscopy.html

Supervised Machine Learning requires Annotated Data

Measured data

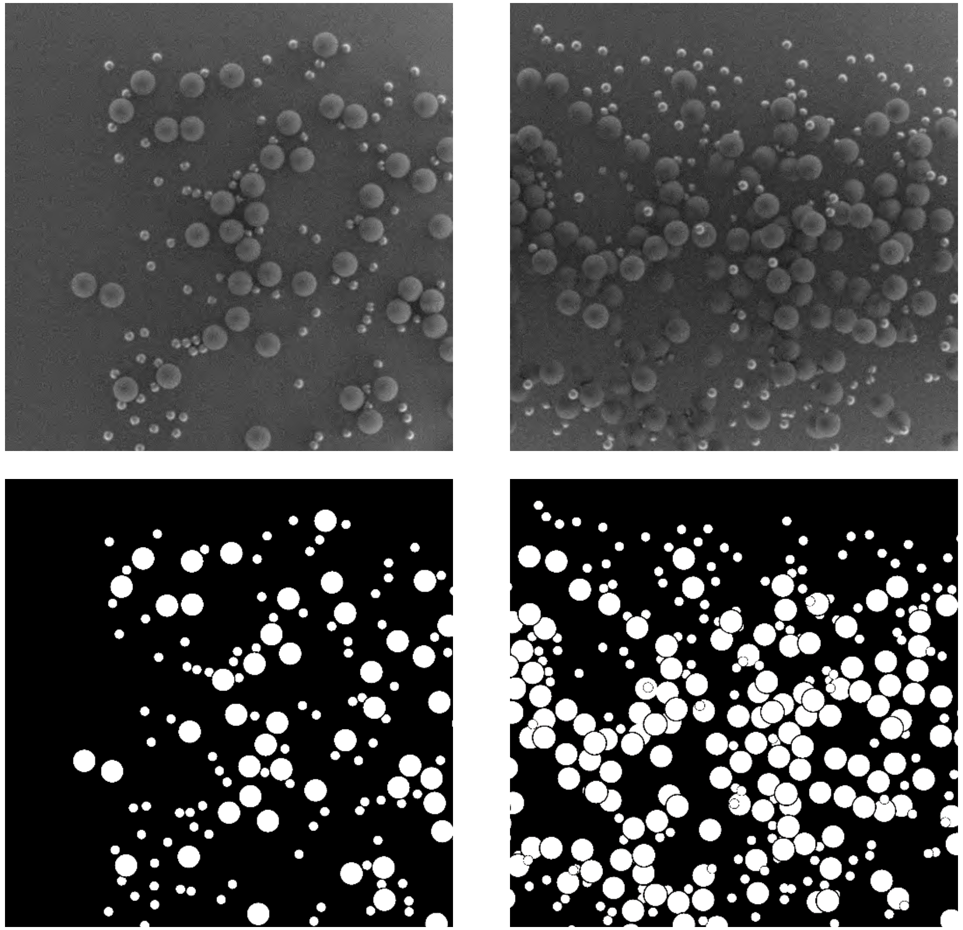


Manually labelled images

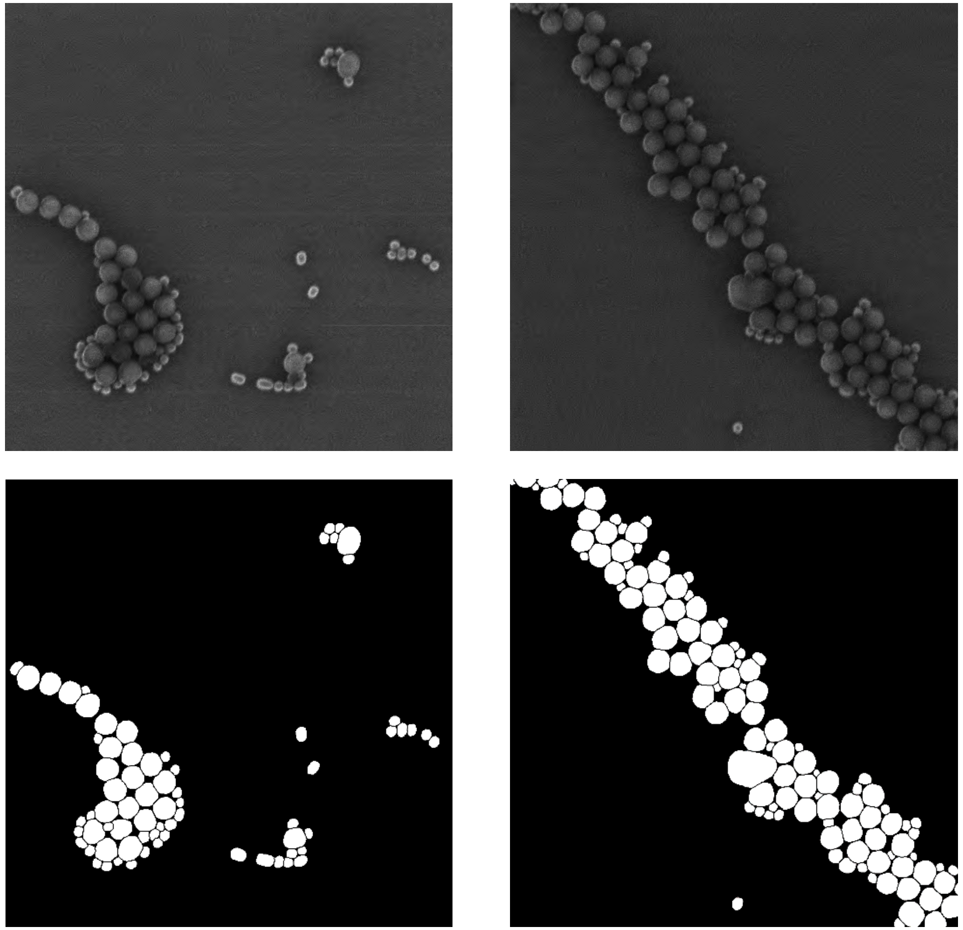


Simulation of SiO_2 Nanoparticle ensembles

Simulated SiO_2 particles



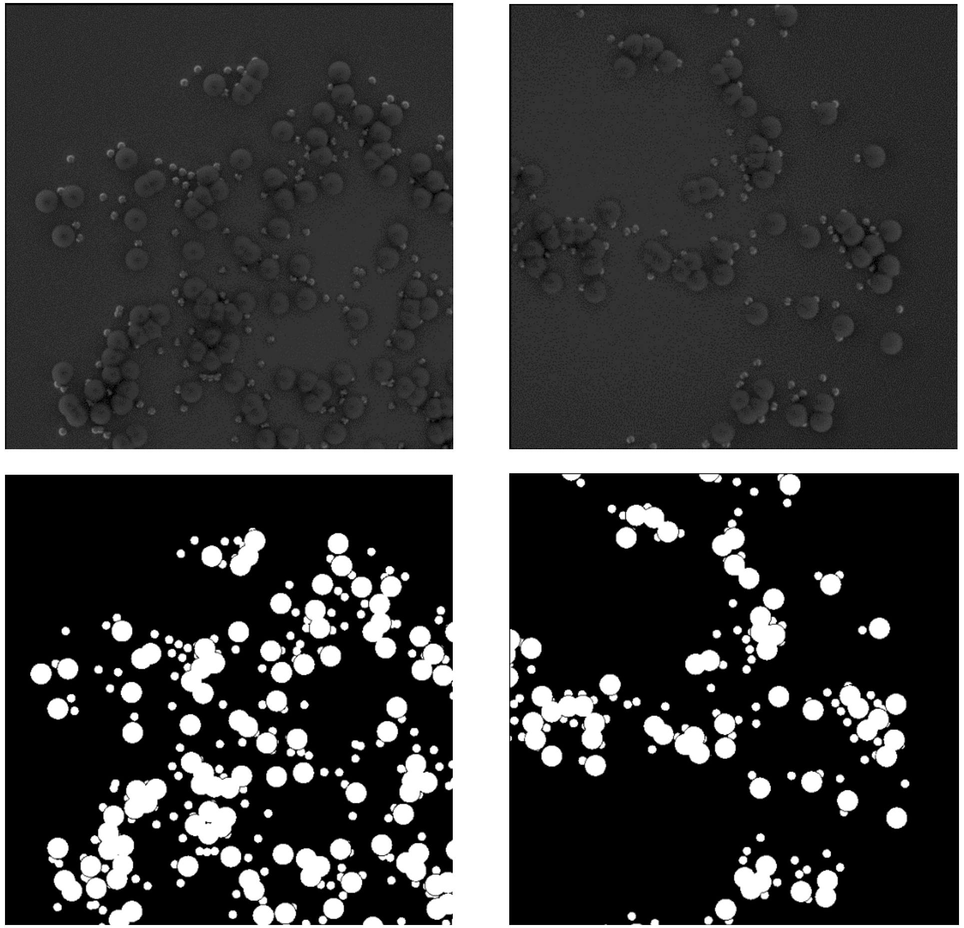
Measured SiO_2 particles



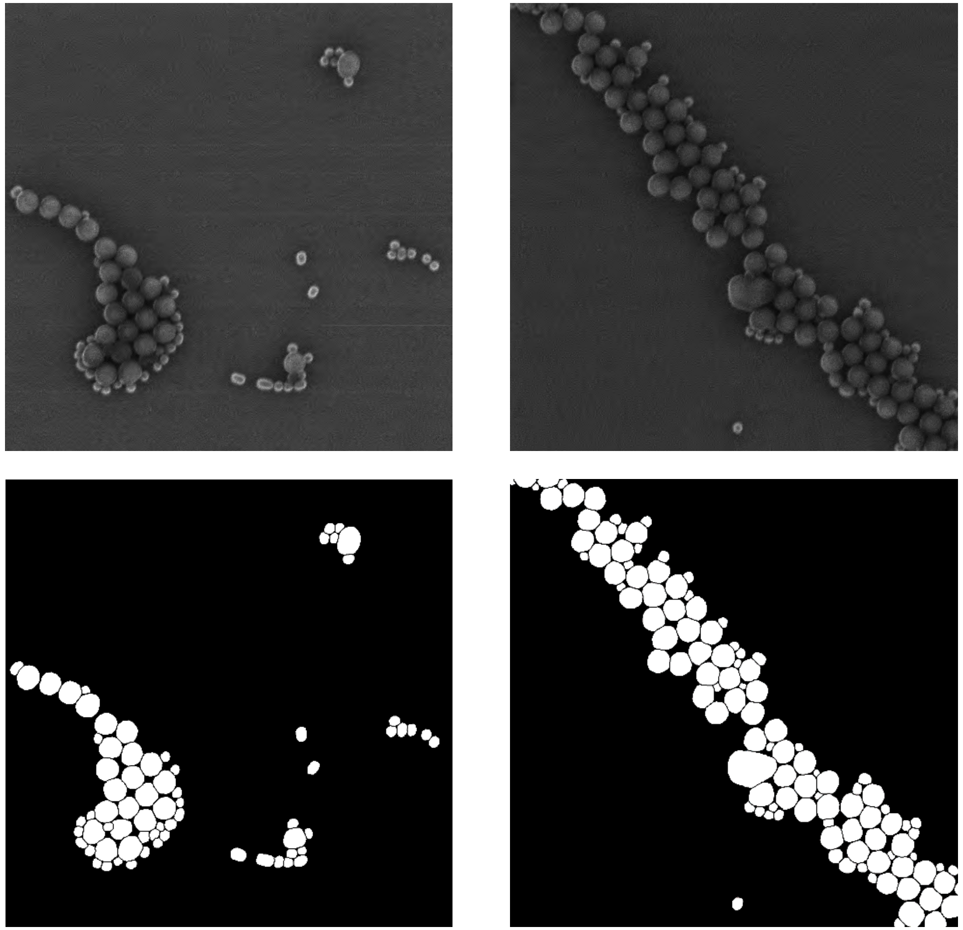
https://www.blender.org/

Simulation of SiO_2 Nanoparticle ensembles

Simulated SiO_2 particles

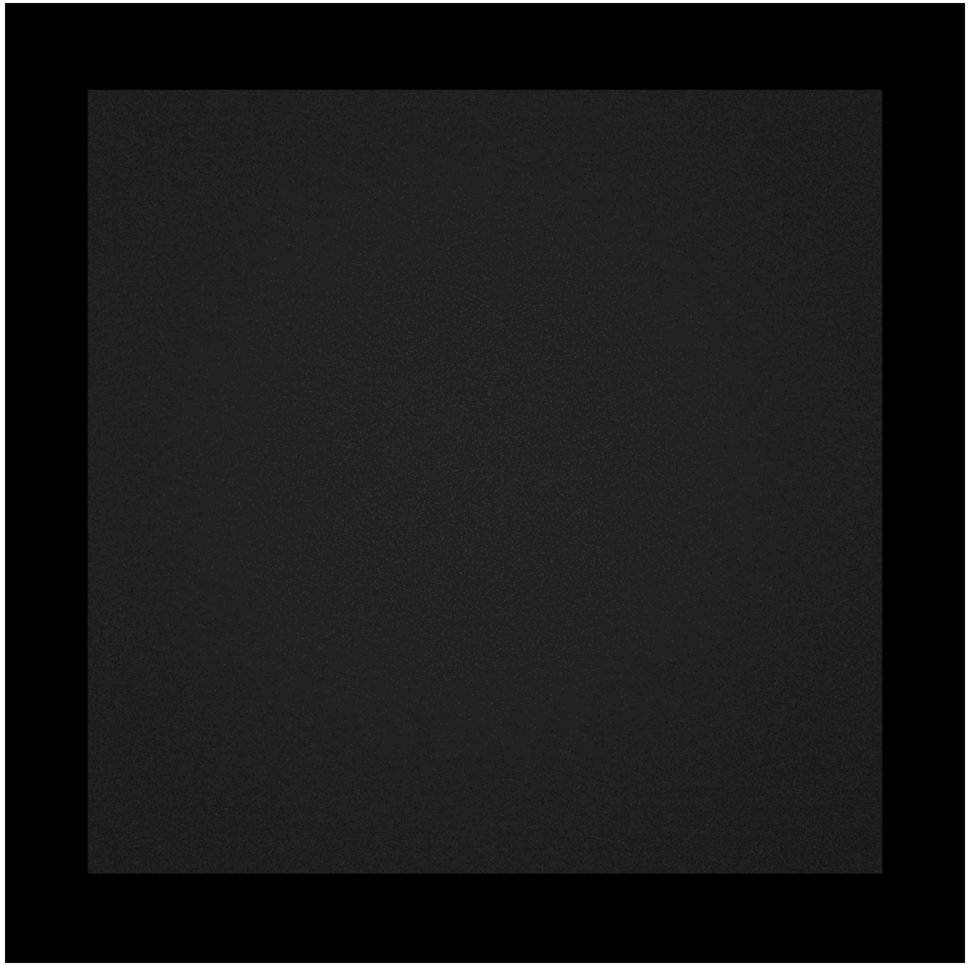


Measured SiO_2 particles



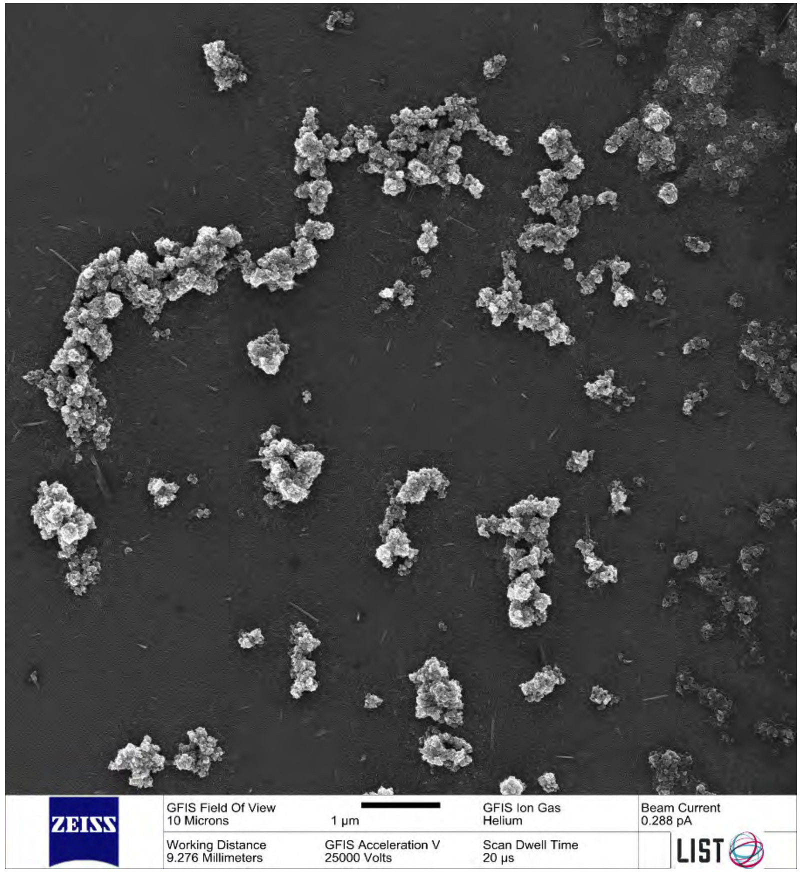
https://www.blender.org/

Simulation of SiO_2 Nanoparticles & their arrangement

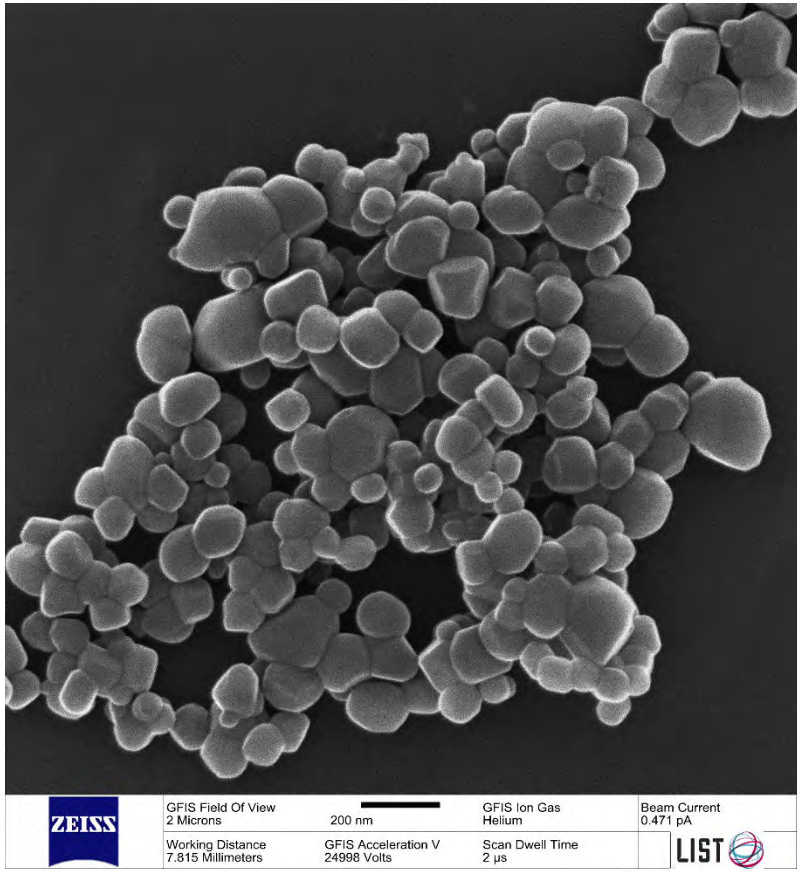


https://www.blender.org/

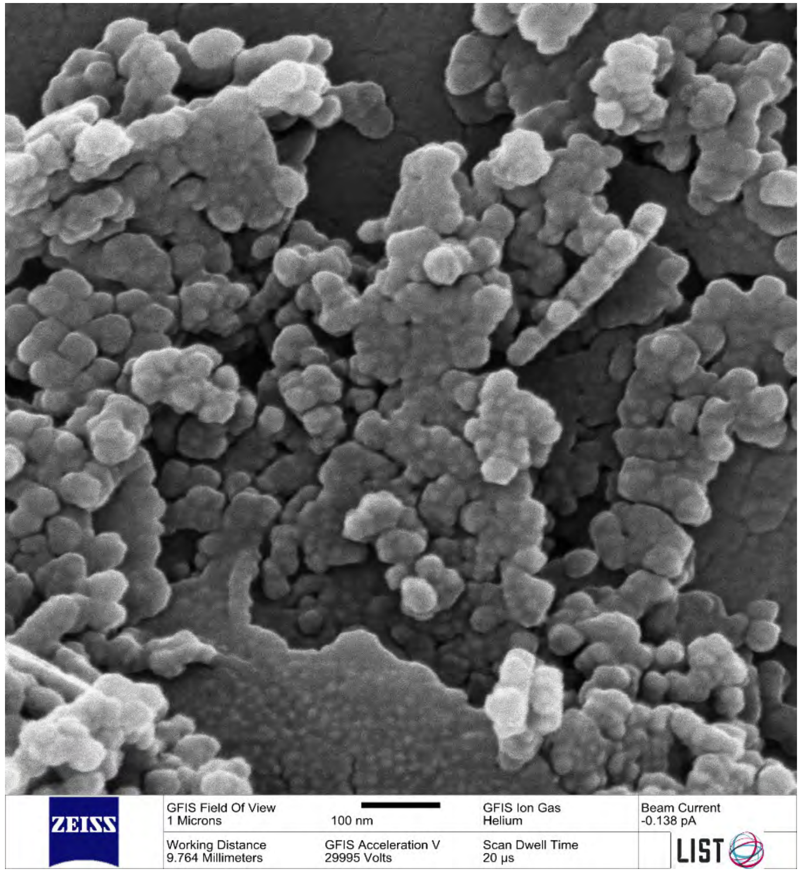
HIM data of different particles



Silver particles

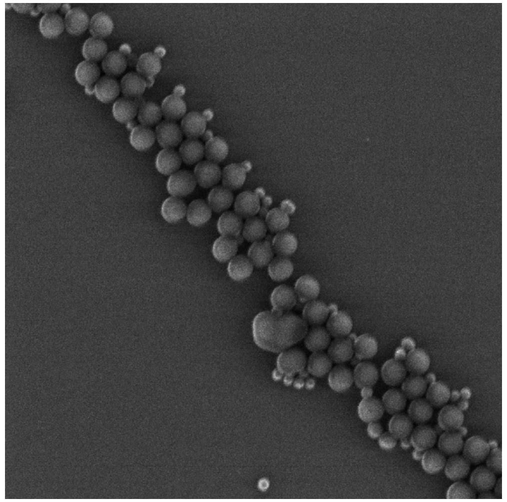


TiO_2 particles

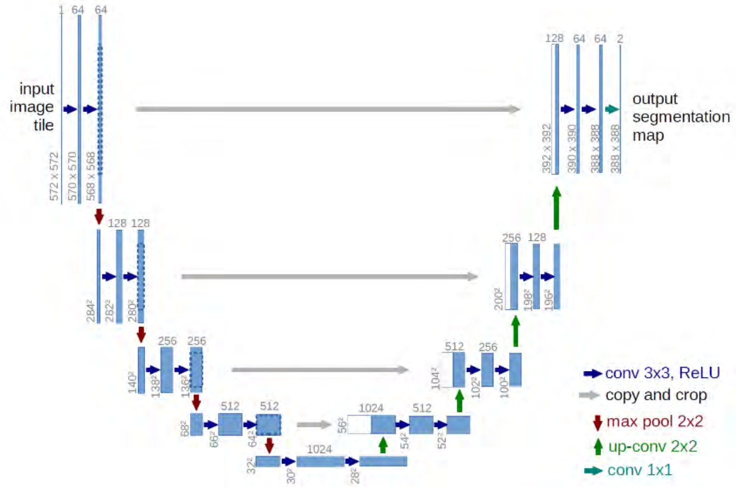


Zinc particles

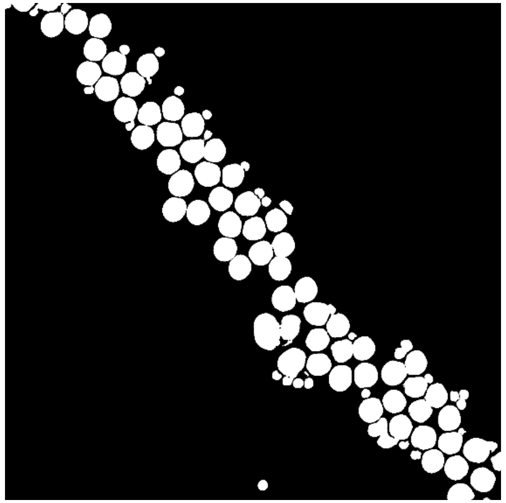
Deep Learning for Particle Segmentation



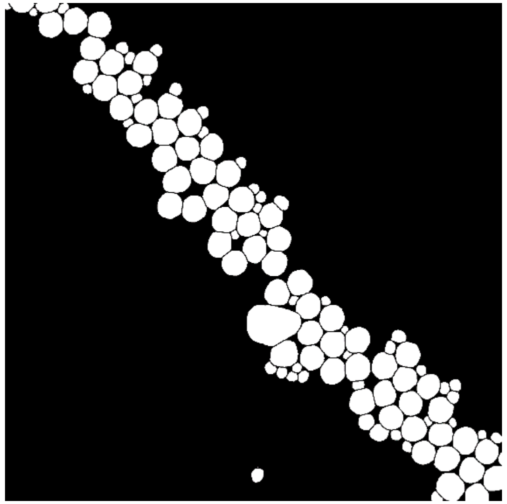
Measured SiO_2 particles



Convolutional Neural Network
(U-Net architecture*)



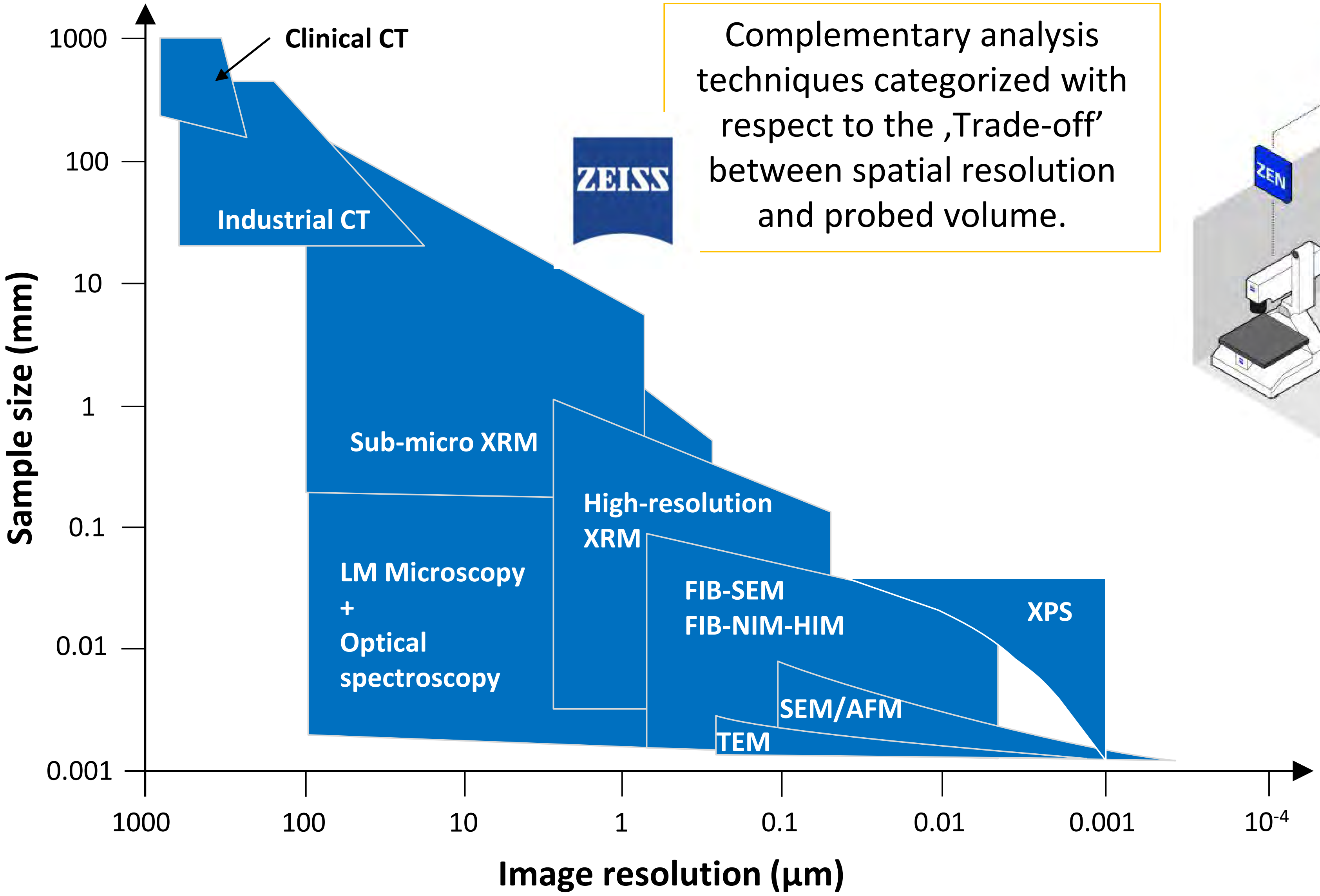
Segmentation by U-Net
trained on simulated images



Segmentation by human

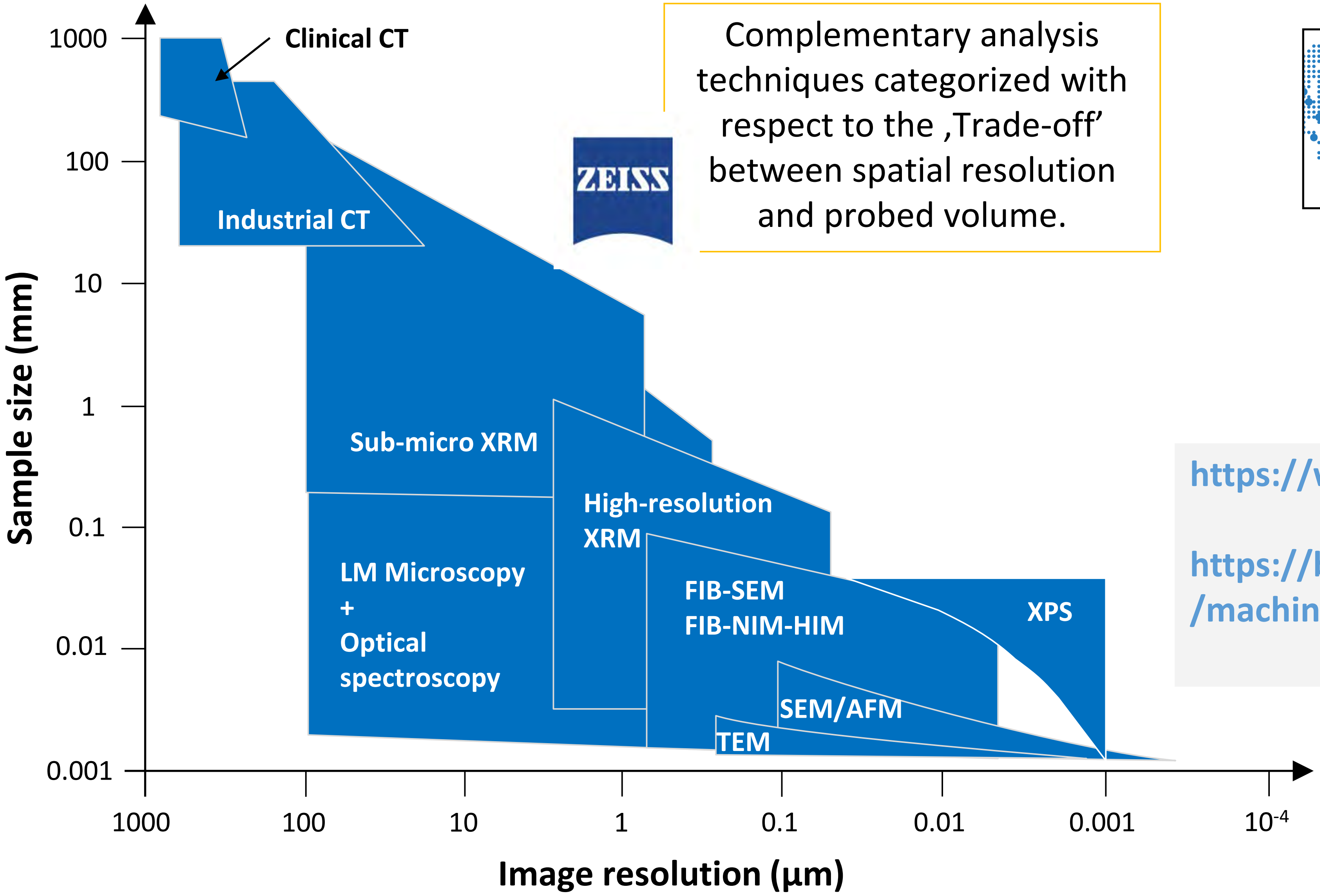
*Ronneberger, Olaf, Philipp Fischer, and Thomas Brox. "U-net: Convolutional networks for biomedical image segmentation." International Conference on Medical image computing and computer-assisted intervention. Springer, Cham, 2015.

Microscopy techniques – context & correlation

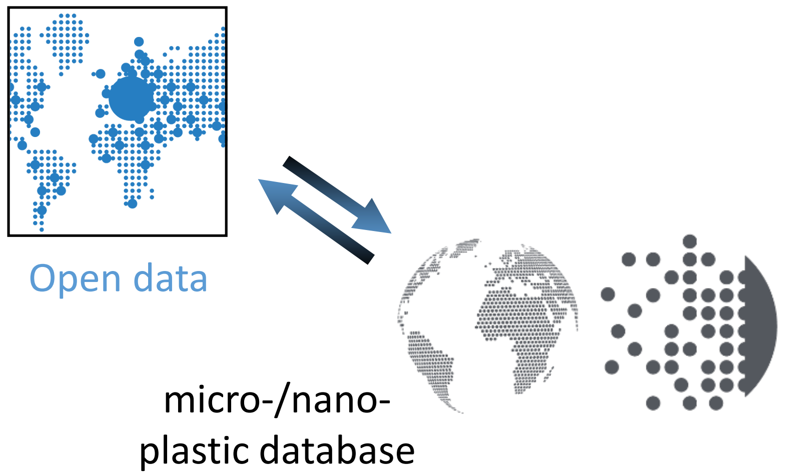


Complementary analysis techniques categorized with respect to the ‚Trade-off' between spatial resolution and probed volume.

Microscopy techniques – context & correlation



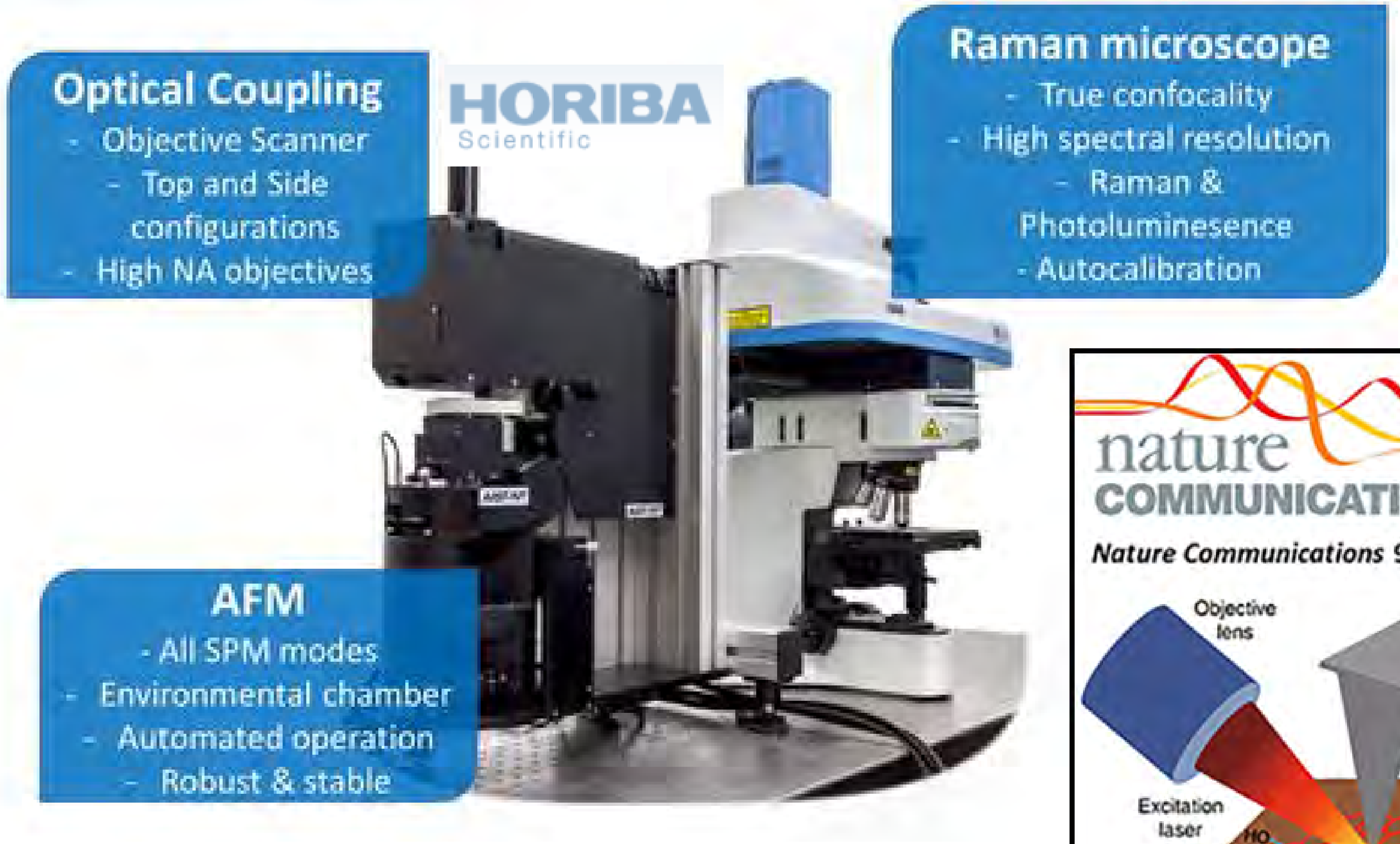
Complementary analysis techniques categorized with respect to the ‚Trade-off' between spatial resolution and probed volume.



https://www.apeer.com/app/#/home

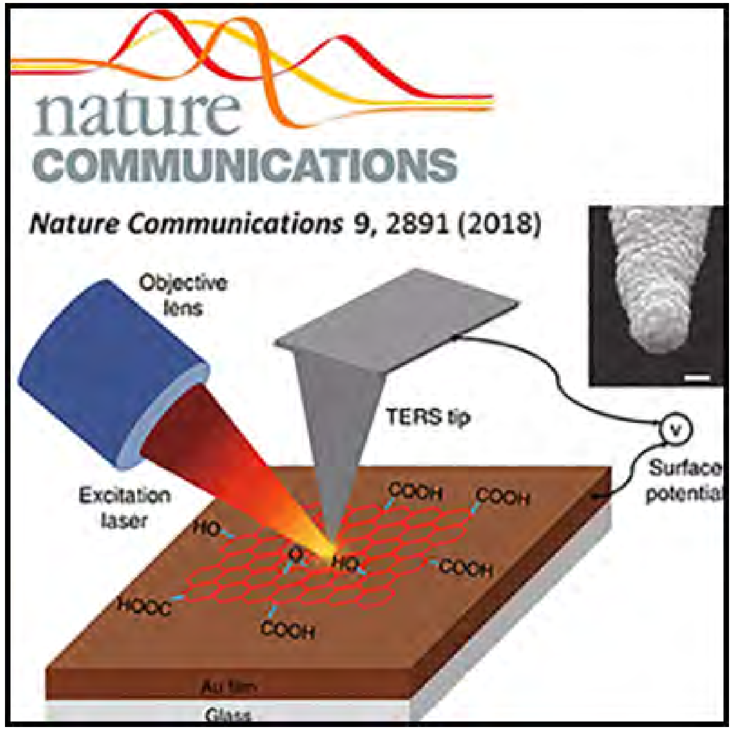
https://blogs.zeiss.com/microscopy/news/en/machine-learning-microscopy/

Raman down to the nano-scale – TERS



TERS with ~ 10 nm resolution:

- multi-modal
- electronic properties + topography + composition



Su et al.
– COOH modified graphene

Thanks for your attention

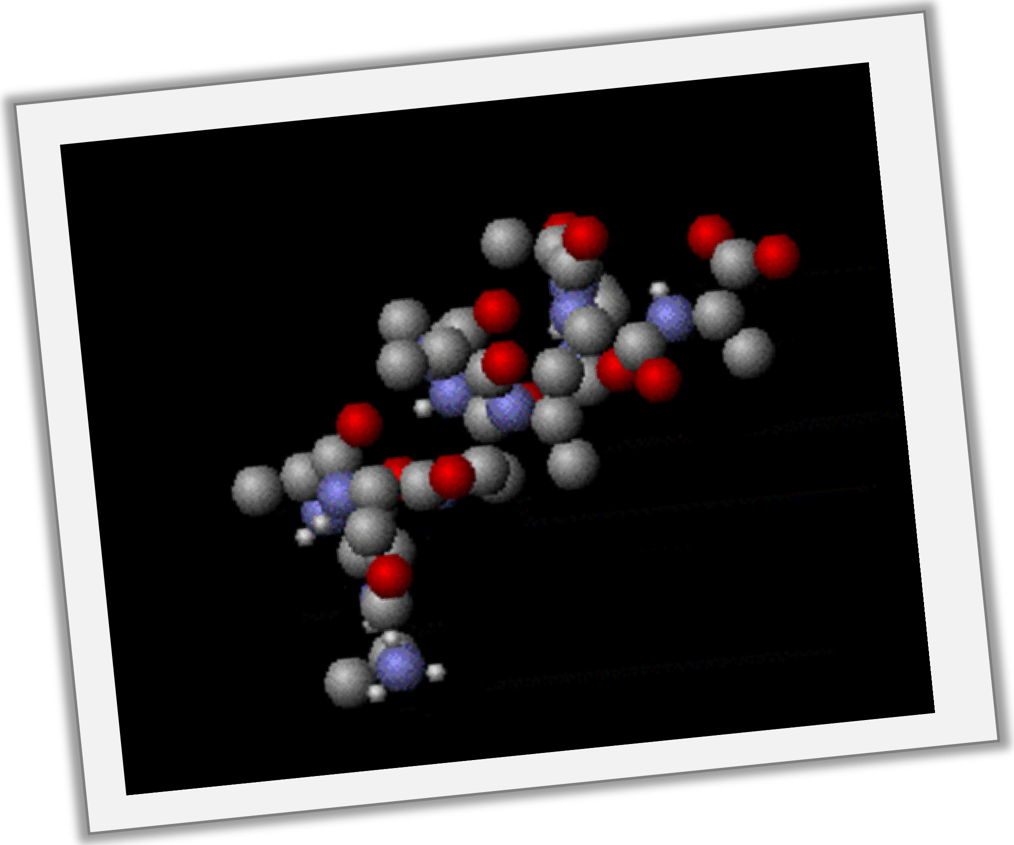
contact:

Tel.: +491796894182

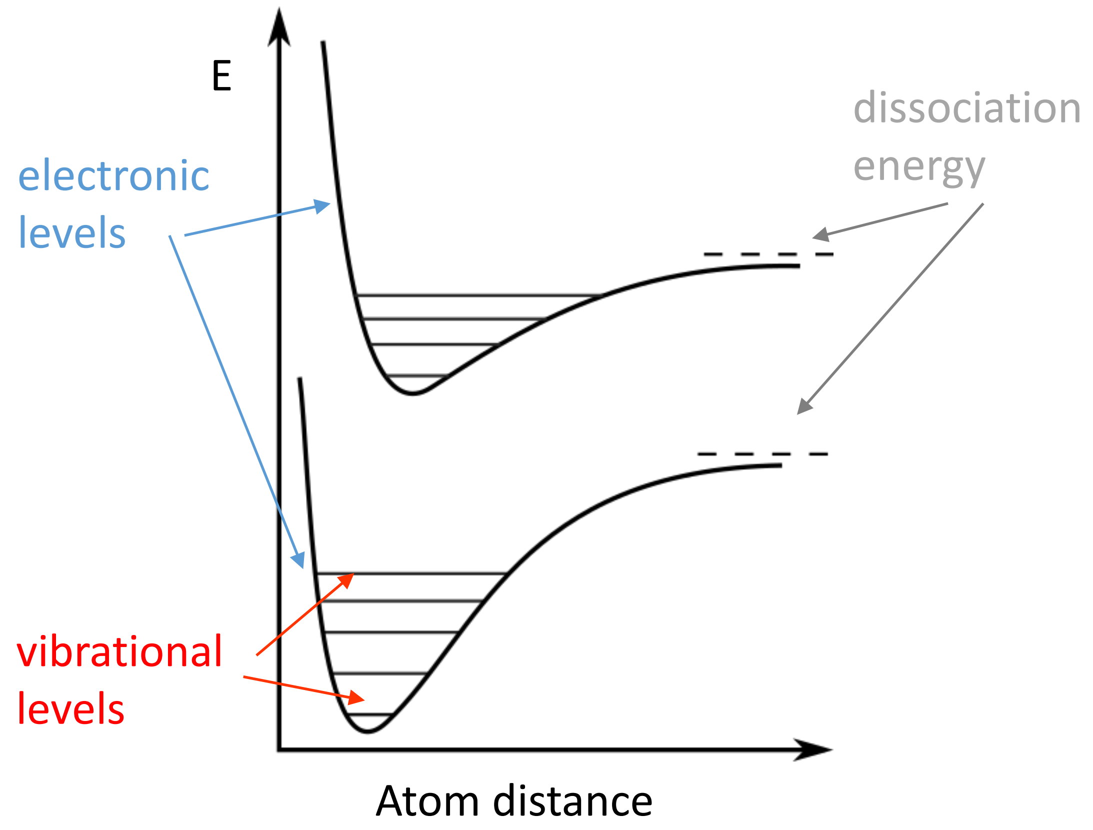
Email: silke.christiansen@mpl.mpg.de

Raman spectroscopy – molecular fingerprinting

Complex organic molecule at room temperature:



Corresponding electronic structure:

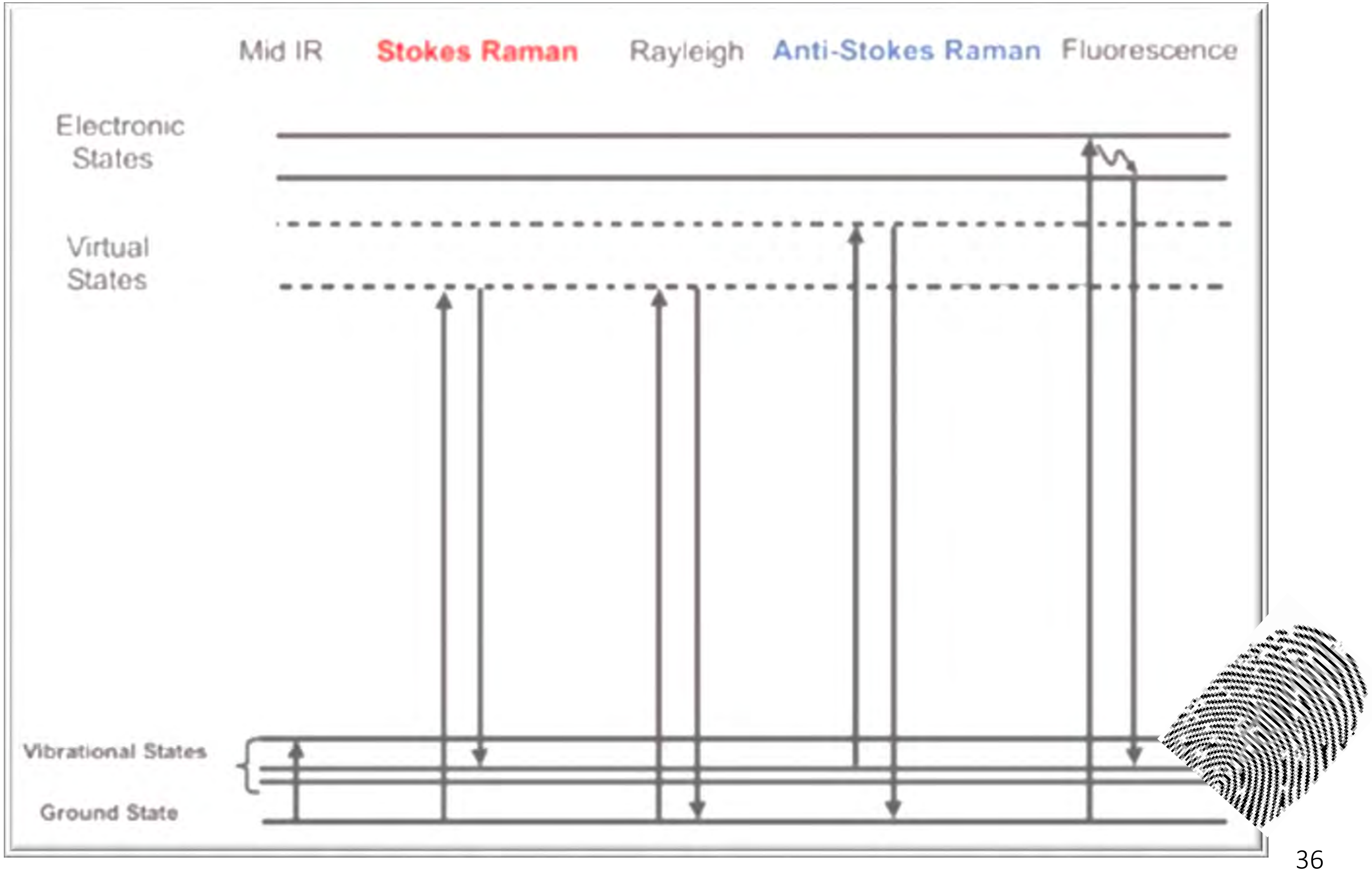


https://www.ozonedepletiontheory.info/ImagePages/thermally-agitated-molecule.html

Jablonski energy diagram

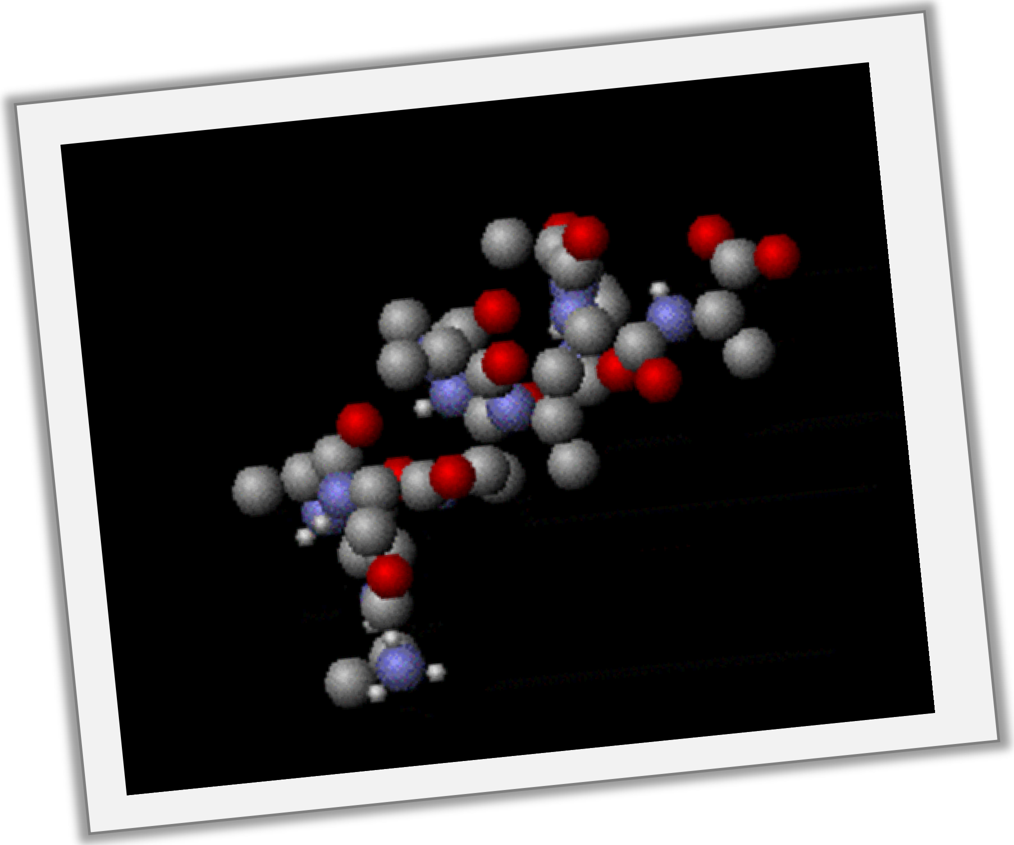
<u>Excitation:</u>
Monochromatic laser light

<u>Inelastic (or Raman) scattering:</u>
of laser light which interacts with molecular vibrations, resulting in the energy of the laser photons being shifted up or down; this is fingerprinting the molecular composition.



Raman spectroscopy – molecular fingerprinting

Complex organic molecule at room temperature:

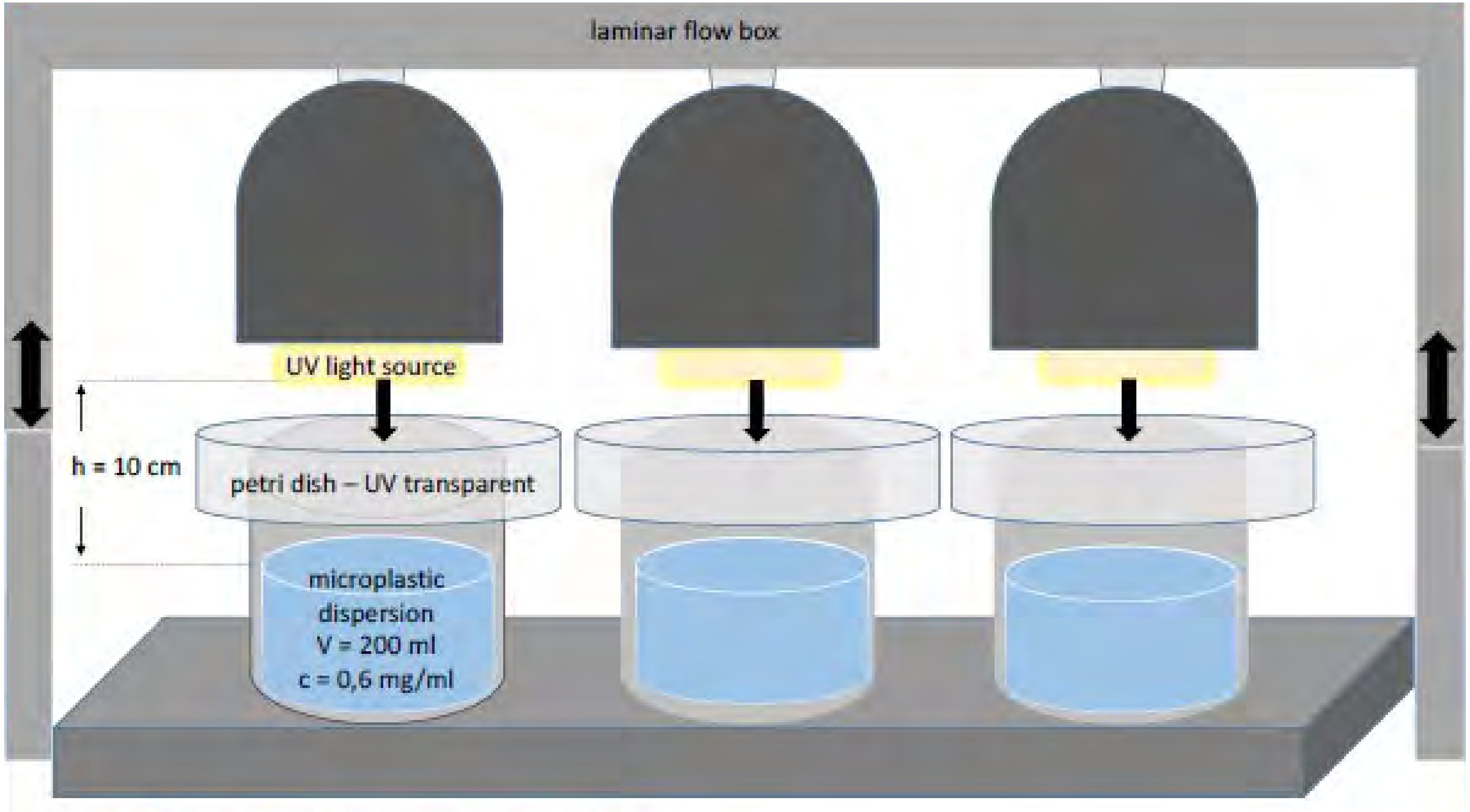


- UV-VIS-NIR lasers are used for Raman excitation
- ≤ 1 µm spot through a microscope objective (max NA 0.9)
- **≤ 1 µm** particle size as compared to **10 µm** of **FTIR**

https://www.ozonedepletiontheory.info/ImagePages/thermally-agitated-molecule.html

G. Sarau, A. Bochmann, R. Lewandowska, S. Christiansen, From micro–to macro–Raman spectroscopy: solar silicon for a case study (Book Chapter - InTech), 221 - 246 (2012)

Wheathering of microplastic



Varying conditions:

- UV light intensity
- water salinity
- polymer materials
- particle size
- particle shape
- Temperature
- weathering duration

K. Oelschlägel, J. Pfeiffer, A. Potthoff, Fraunhofer Institute for Ceramic Technologies and Systems IKTS, Dresden, Germany, unpublished

Our research home

INAM

INAM

Innovations-Institut
für Nanotechnologie und
korrelative Mikroskopie

… a new location for correlative microscopy in close collaboration with …

MPL

ZEISS

HZB Helmholtz Zentrum Berlin

Fraunhofer IKTS

Filter membrane selection

INAM

Material of the membrane filter	Smooth and unstructured surface	Applicability of Particle Finder module		Raman background of the filter material		Maximum laser power for non-destructive Raman measurement of all standard particles (mW)	
		Illumination		Laser wavelength			
		Bright field	Dark field	532 nm	785 nm	532 nm	785 nm
Regenerated Cellulose	No	Not tested[a]					
Nitrocellulose	No						
Cellulose acetate	No						
Aluminium oxide	No						
Silver	No						
PC white	Yes	No	No	Intensive Raman spectrum		Not tested[a]	
PC black	Yes	No	Yes	Intensive Raman spectrum		Not tested[a]	
PC coated with gold	Yes	No	Yes	Weak fluorescence		~1.2	~5.3
PC white coated with titan	Yes	No	Yes	Burning		Not possible[b]	
PC white coated with nickel	Yes	No	Yes	No background	Burning	~1.2	Not possible[b]
PC white coated with aluminium	Yes	No	Yes	No background	Weak fluorescence	~3.2	~5.3

[a] Material was excluded from further tests because of prior results

[b] Non-destructive Raman measurement was not possible due to burning of particles

PC Polycarbonate

- Pore size < 1 µm
- Smooth and unstructured surface for good visibility of particles ≤ 1 µm
- High optical contrast for automatic particle detection
- No spectral interference from substrate (Raman, Fluorescence)
- No burning of particles with one or both lasers

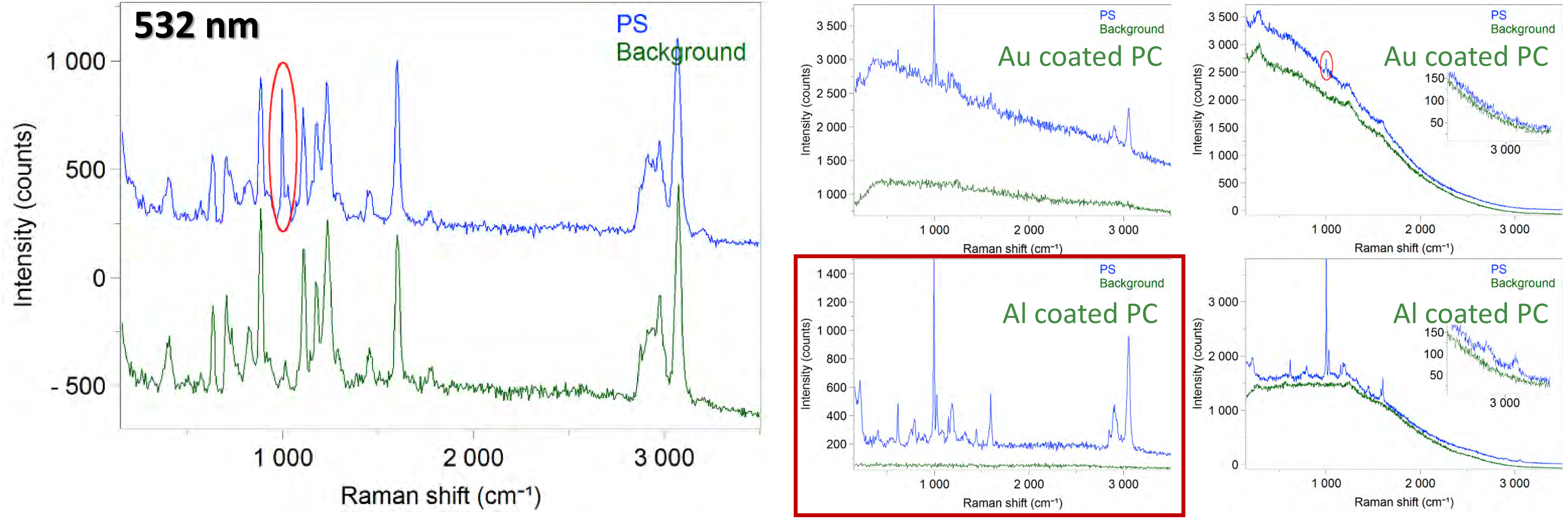
B. E. Oßmann, G. Sarau, S. W. Schmitt, H. Holtmannspötter, S. Christiansen, W. Dicke, Analytical & Bioanalytical Chemistry **409,** 4099 (2017)

Effect of metal coating and laser

Polystyrene (PS) on Polycarbonate (PC) filters

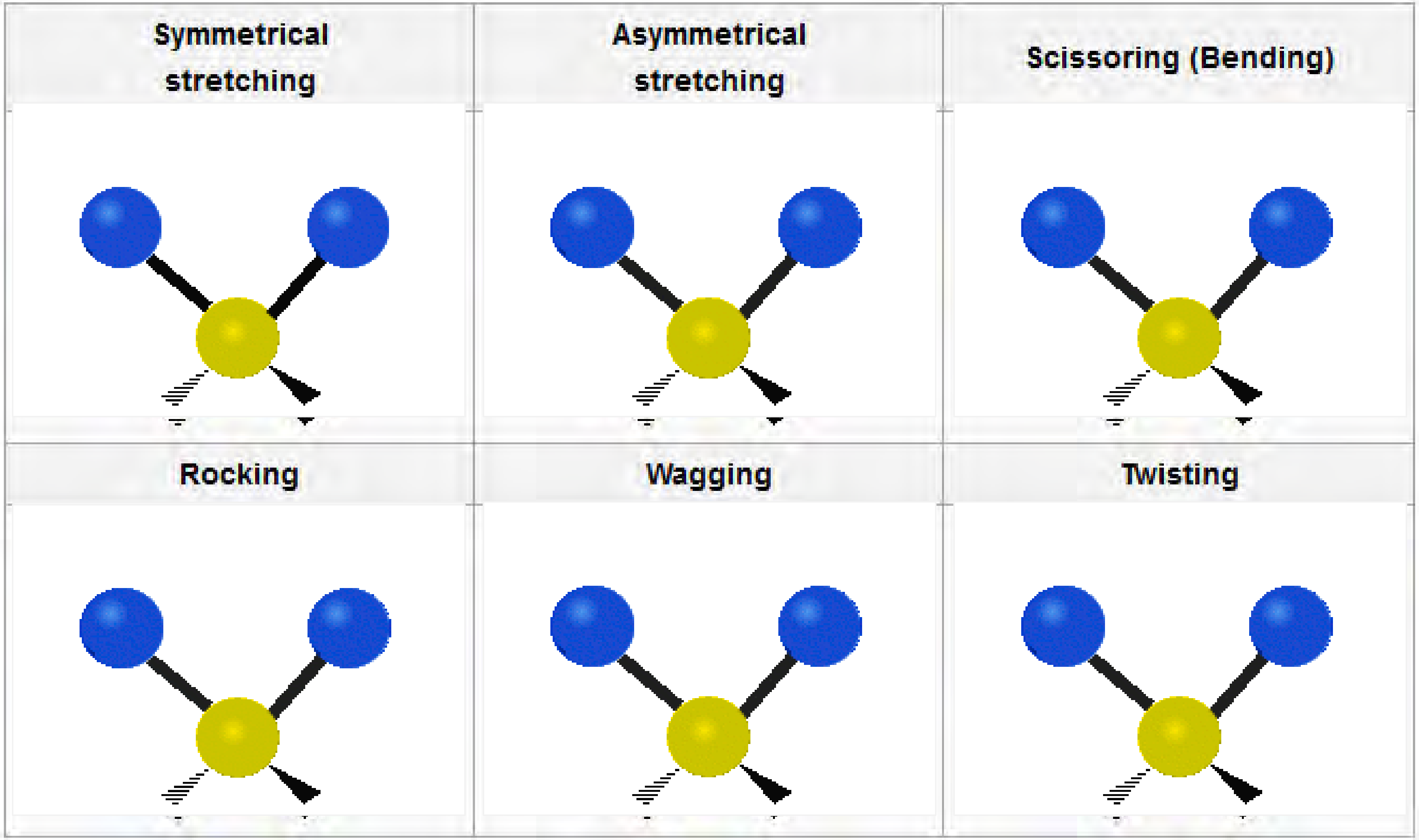
Au vs Al at 532 nm

Au vs Al at 785 nm



- ≤ 1 µm PS used as plastic particle standard
- Pronounced Raman background from PC membrane filter
- Only the C-H bending of PS at 1000 cm^{-1} can be separated
- Background, weak/less Raman peaks on Au for both lasers
- No background, intense Raman peaks on Al for 532 nm
- Background, weak/less Raman peaks on Al for 785 nm

Vibrational modes of – CH_2 group



https://en.wikipedia.org/wiki/Molecular_vibration

Micro-/Nano-plastics in mineral water

Sample pre-treatment

- Remove labels, clean outside of the bottles, transfer to cleaned Erlenmeyer flasks
- Add EDTA to dissolve Ca, $MgCO_3$
- tenside – lower surface tension for improved particle suspension & homogeneity

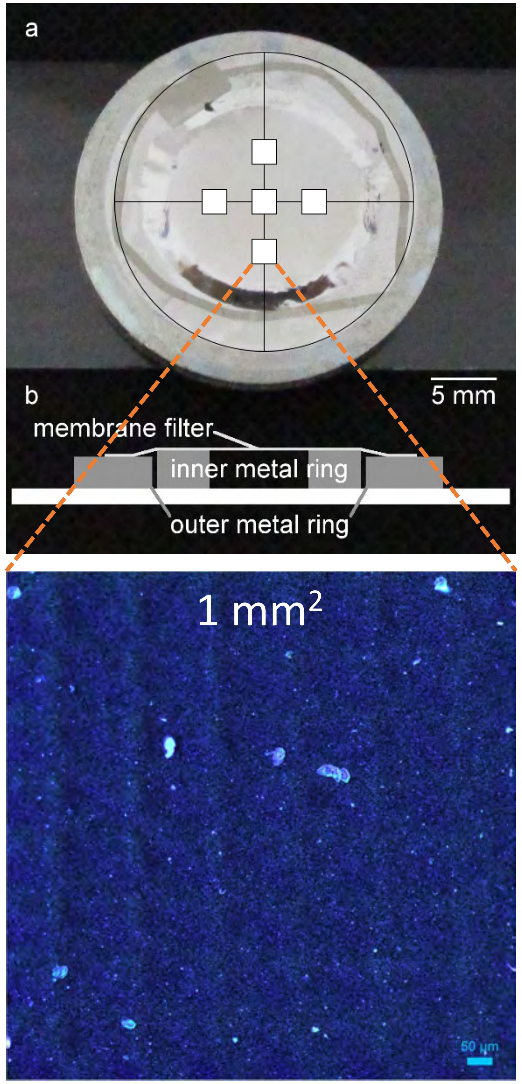
- glass
- PET single use
- PET reusable

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Micro-/Nano-plastics in mineral water

Measure-ment

- Filtration of aliquot (250 ml) through Al coated PC membrane filters via vacuum
- Evaluation of surface area (5 x $1 mm^2$ in centre out of $113\ mm^2$)
- Dark field optical microscopy (50x)
- Automated particle detection
- Automatic Raman measurement

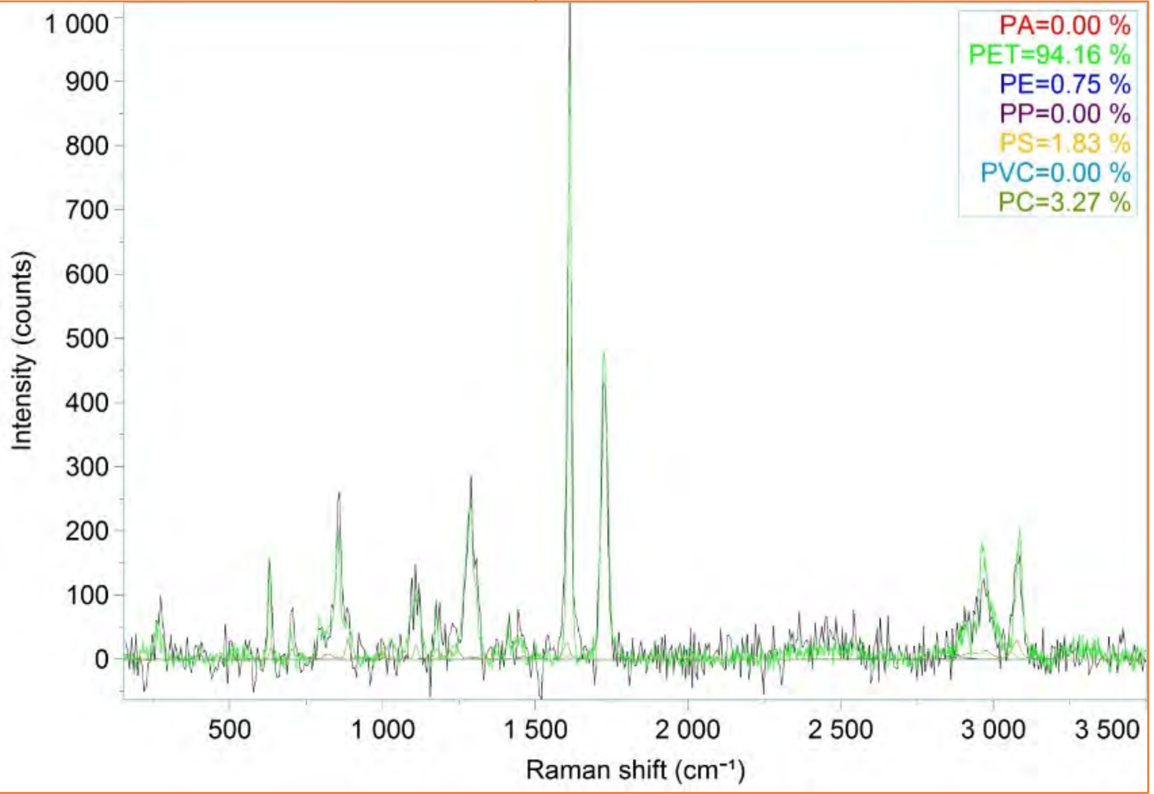


B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Micro-/Nano-plastics in mineral water

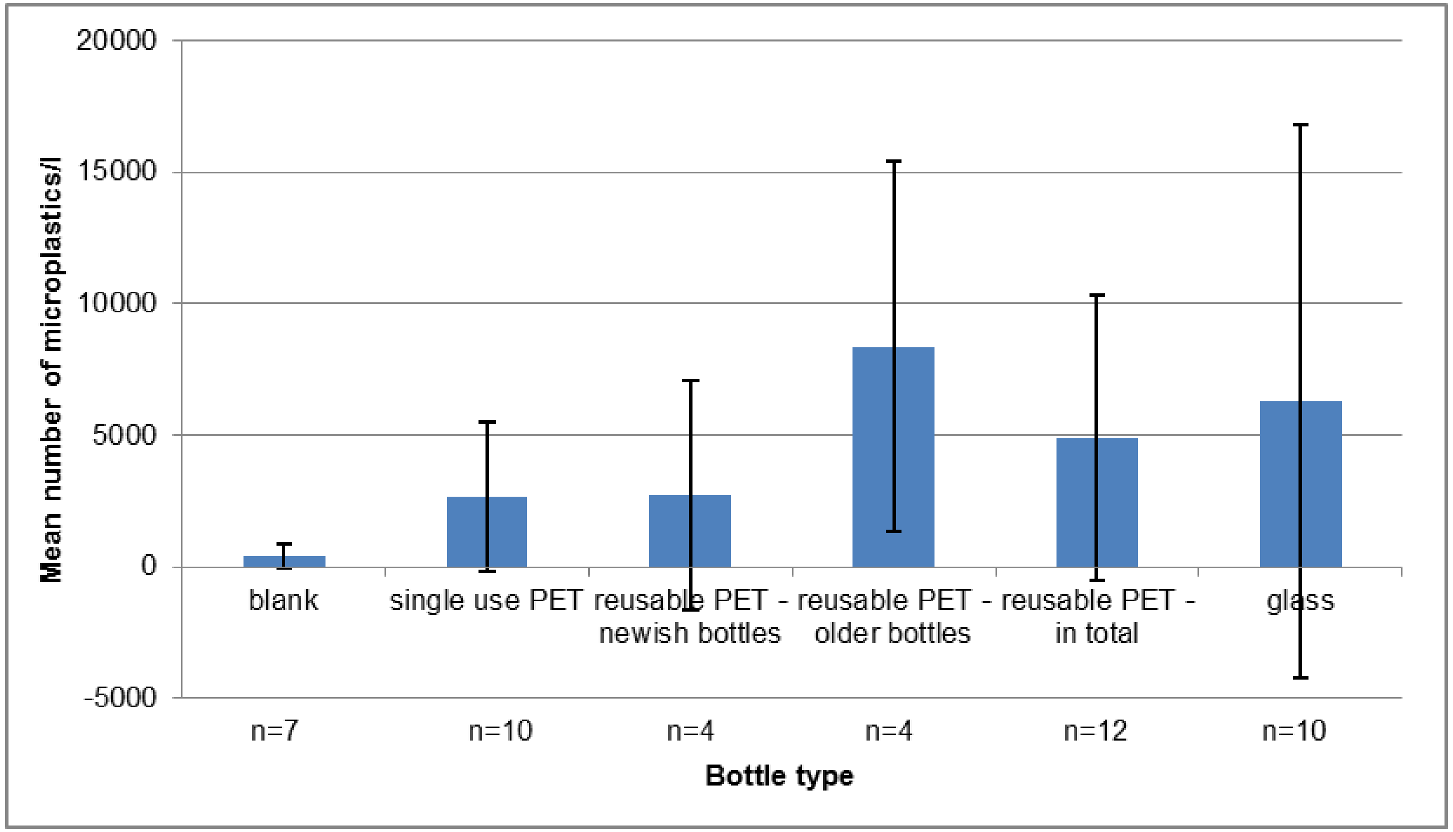
Data Analysis

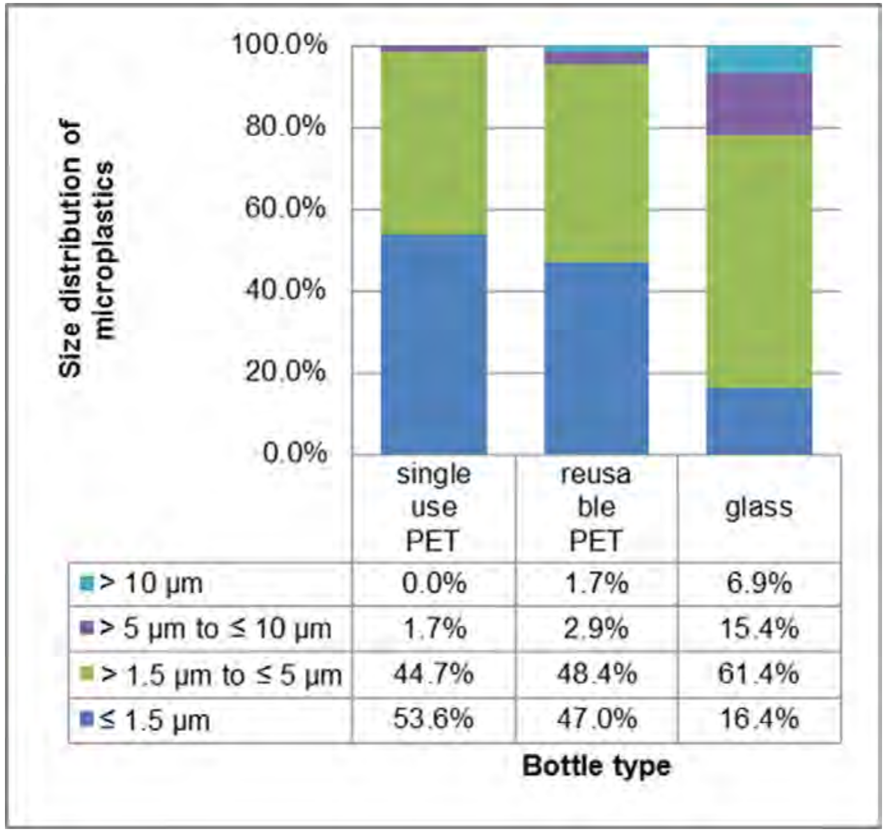
- Processing of Raman spectra
- Screening for polymers with CLS algorithm
- Comparison with database
- Manual remeasurement (if necessary)



B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Number, size and type of micro-/nano-plastics

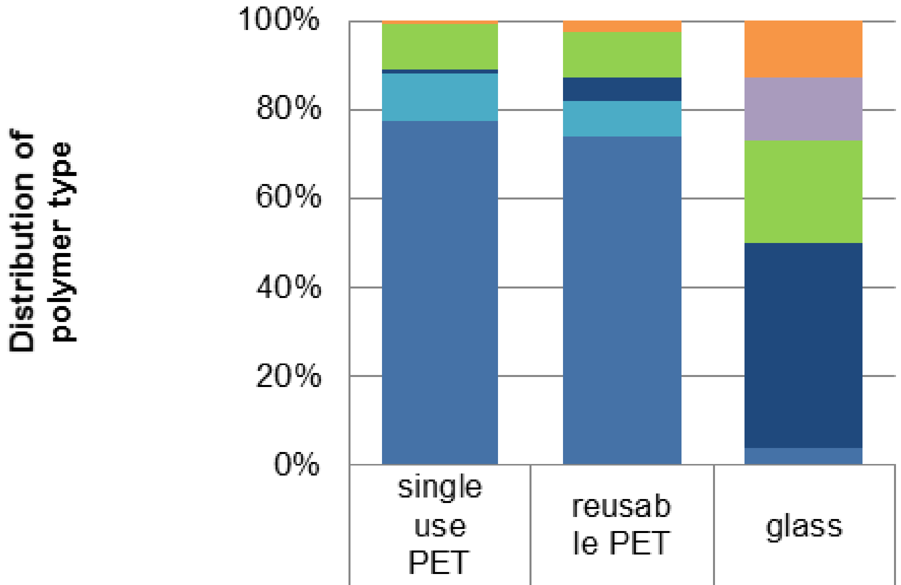




	single use PET	reusable PET	glass
> 10 µm	0.0%	1.7%	6.9%
> 5 µm to ≤ 10 µm	1.7%	2.9%	15.4%
> 1.5 µm to ≤ 5 µm	44.7%	48.4%	61.4%
≤ 1.5 µm	53.6%	47.0%	16.4%

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Number, size and type of micro-/nano-plastics



	single use PET	reusable PET	glass
others	1.0%	2.6%	13%
Styrene-Butadiene-Copolymer			14%
PP	10%	10%	23%
PE	0.7%	5.4%	46%
PET+olefin	11%	7.7%	
PET	78%	74%	3.6%

Bottle type

- 32 samples from 21 brands
- PET bottles: age determines PET contamination
- small-size PET particles dominant:
  - ~95 % ≤ 5 µm
  - ~50 % ≤ 1.5 µm
- Glass bottles: abrasion of caps (PP, PE) and cleaning machine parts (PS, SBC)

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Number & size of micro-/nano-plastics in mineral water

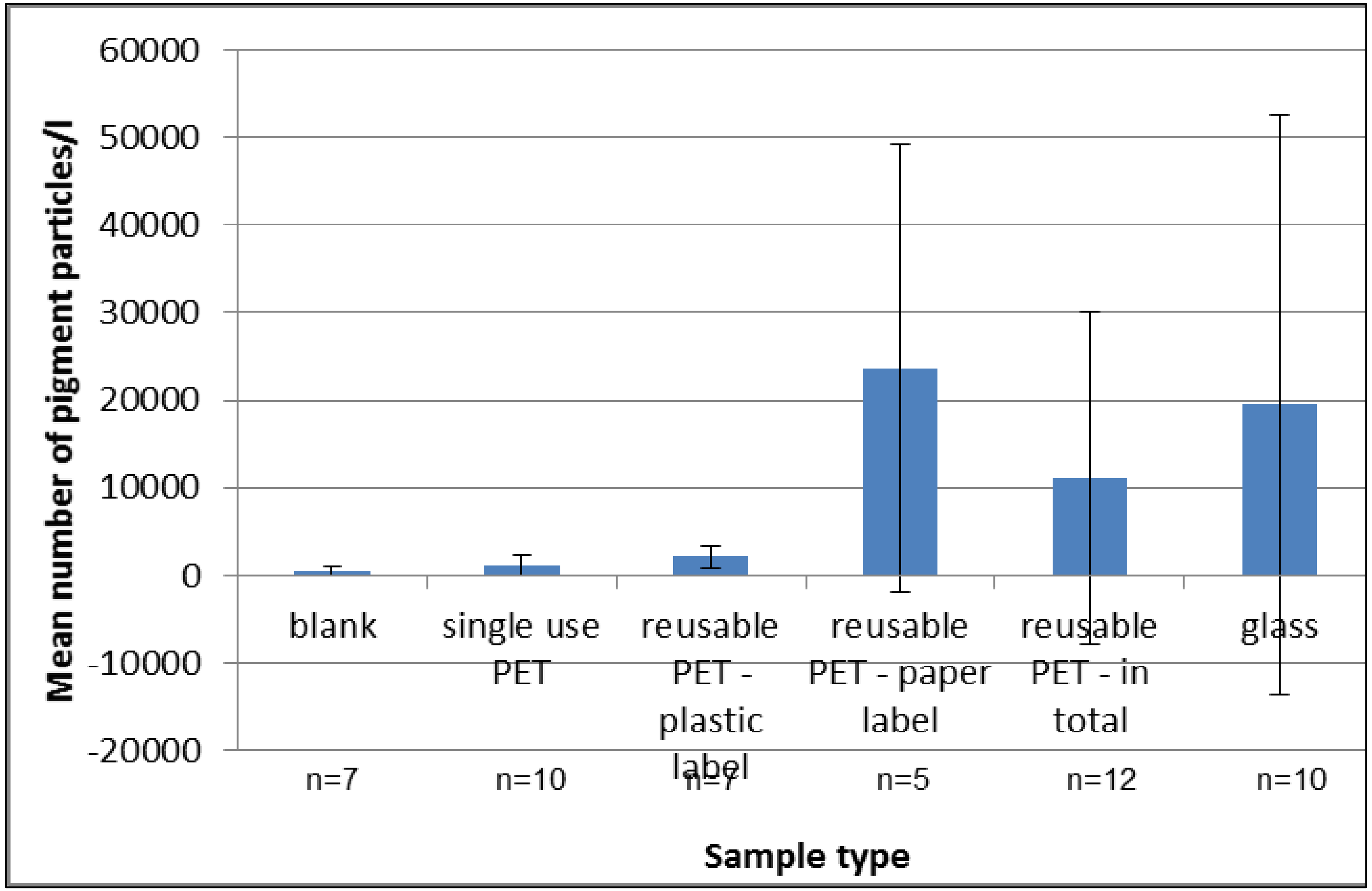
	Microplastics/l								
	single use PET			reusable PET			glass		
	Oßmann et al. (2018)		Schymanski et al. 2018)	Oßmann et al. (2018)		Schymanski et al. (2018)	Oßmann et al. (2018)		Schymanski et al. (2018)
≤1.5 µm	1419±1614			2298±3048			1031±1773		
>1.5 µm to ≤5 µm	1184±1329			2365±2457			3860±1746		
>5 µm to ≤10 µm	45±64	45±64	14±14	143±226	226±307	118±88	967±1779	1401±2588	50±52
>10 µm	0±0			83±136			434±715		
total	2648±2857			4889±5432			6292±10521		

38 bottles: **D. Schymanski**, C. Goldbeck, H. U. Humpf, P. Fürst, Water Research 129 154-162 (2018)

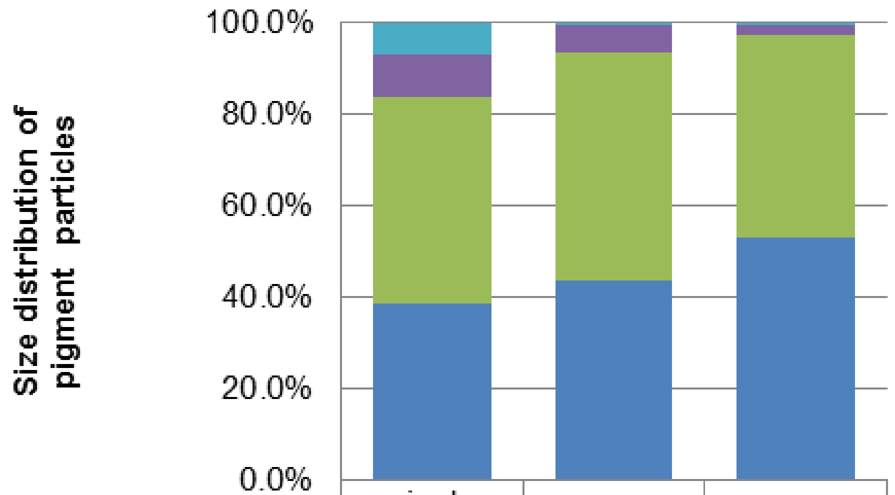
32 bottles: **B. E. Oßmann**, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Number, size and type of pigments

Number of pigment particles



Size of pigment particles

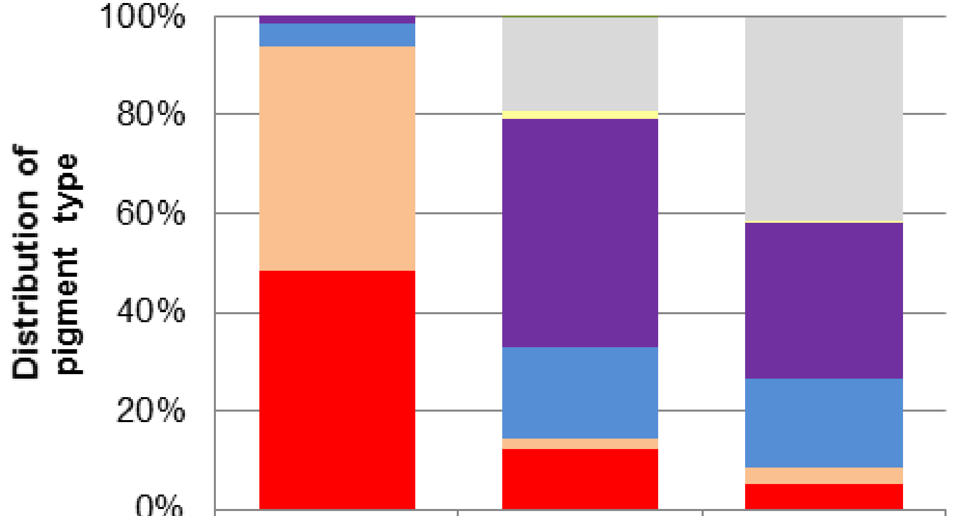


	single use PET	reusable PET	glass
> 10 µm	6.9%	0.5%	0.4%
> 5 µm to ≤ 10 µm	9.2%	6.1%	2.3%
> 1.5 µm to ≤ 5 µm	45.4%	49.6%	44.2%
≤ 1.5 µm	38.5%	43.8%	53.1%

Bottle type

B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

Number, size and type of pigments

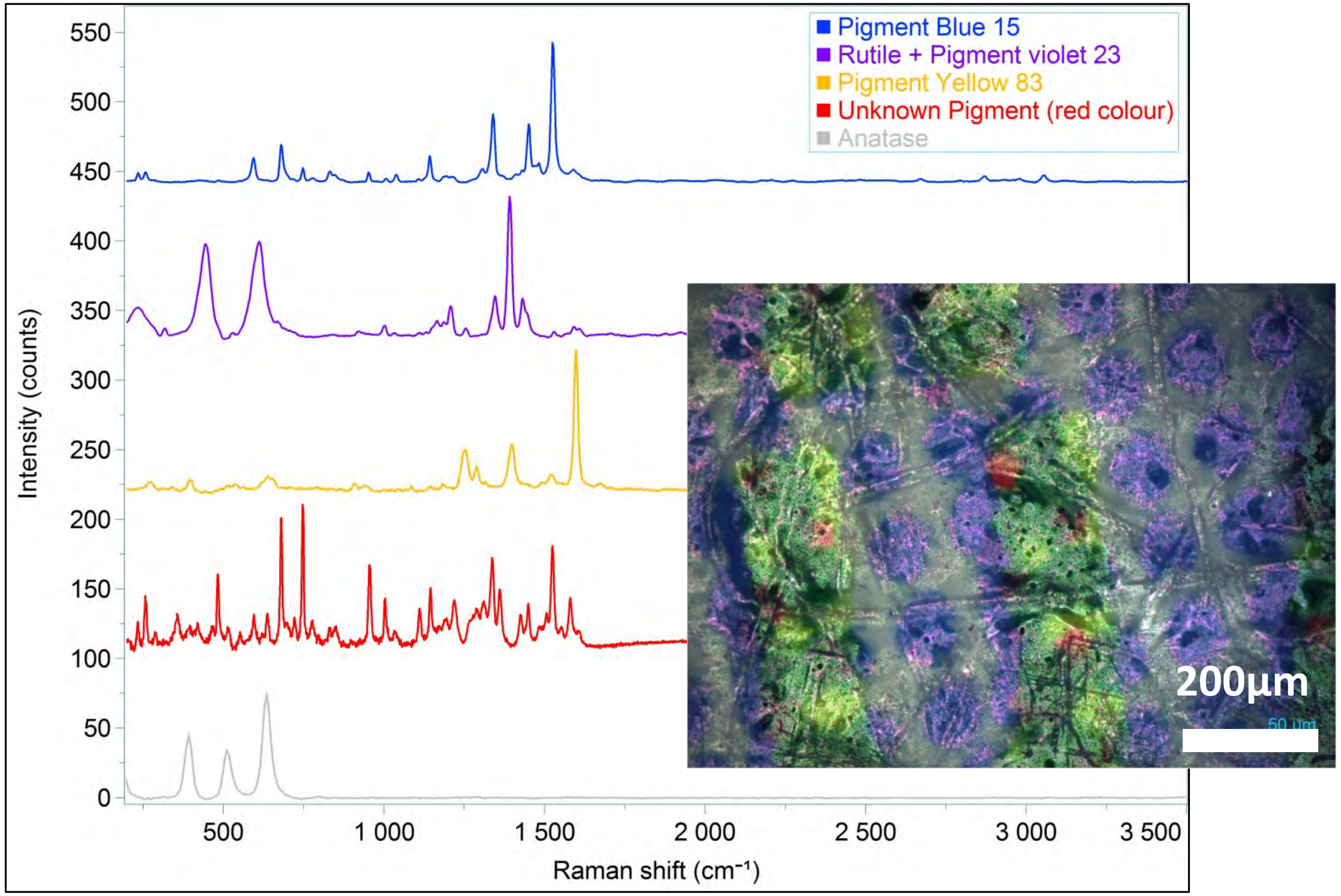


	single use PET	reusable PET	glass
indigo		0.1%	
mixture		19%	42%
PY83		1.5%	0.4%
PV23	1.5%	46%	31%
PB15	4.6%	19%	18%
rutile	45%	2.2%	3.1%
anatase	49%	12%	5.2%

Bottle type

in 1l

Raman spectra of pigments on lable – different types

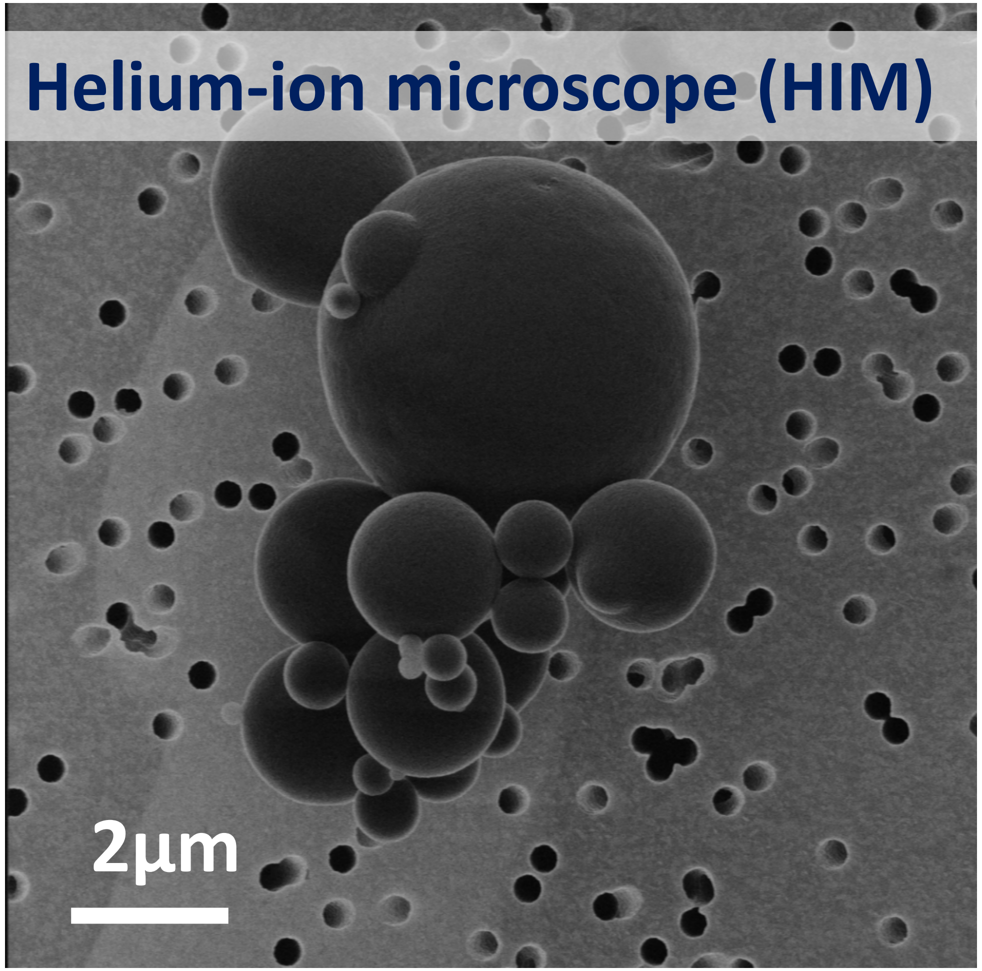


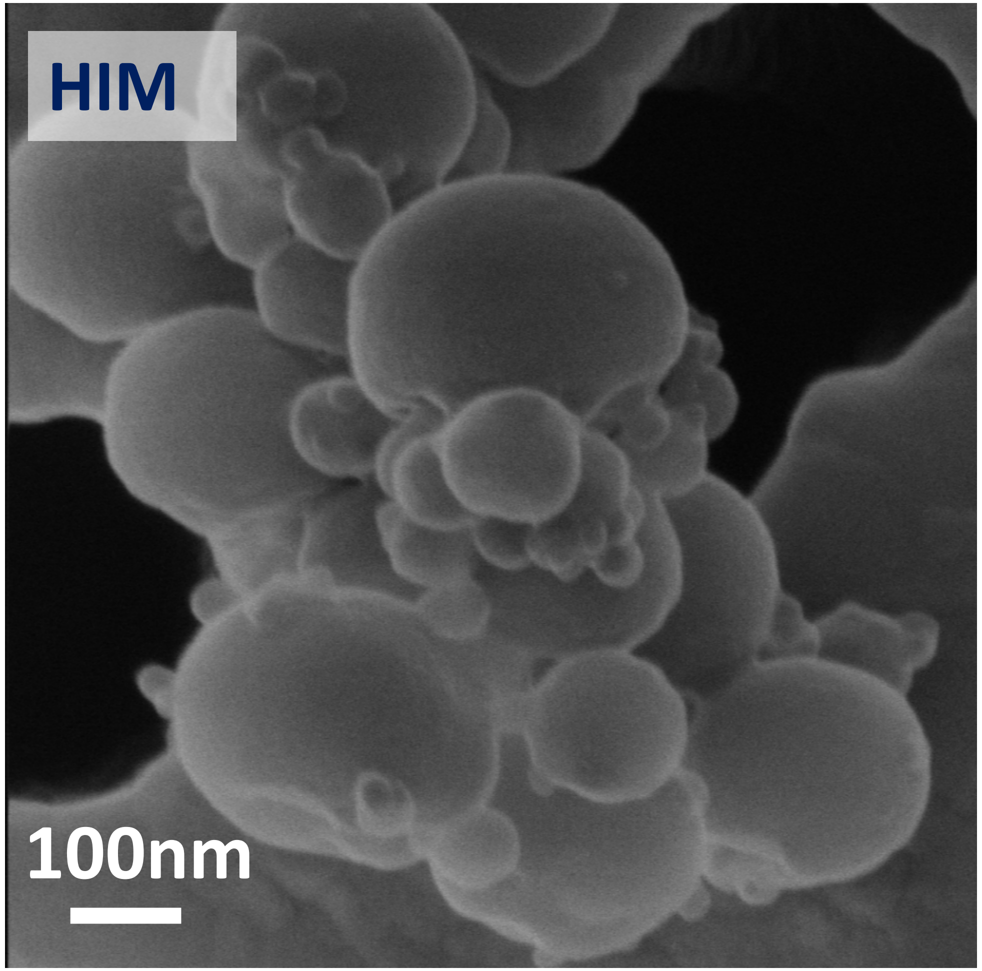
B. E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S. Christiansen, W. Dicke, Water Research **141** 307-316 (2018)

conclusions

- Our correlative microscopy / spectroscopy workflows open the way towards standardized microplastic monitoring methods
- Detected microplastic and pigment particles are > 90 % ≤ 5 µm and > 40% ≤ 1.5 µm, which is highly important because of toxicological risks after oral ingestion via food
- In mineral water samples, not only the PET packing itself, but also the cleaning of reusable PET and glass lead to contamination with microplastics
- Pigment particles from printed paper labels and additives from PET material are an addition source of contamination
- Material properties of microplastics are continuously changing due to weathering

Nano-plastic particles on PC filter membrane





B. Oßmann, G. Sarau, L. Kling, G. Sarau, S. Christiansen, unpublished