Recommendations for testing laboratories on achieving reliable identification and characterisation of microplastics with LD-IR

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Microplastics pollution

- Over ***350 million tonnes of plastic waste*** are produced globally every year
- Once released to the environment plastics fragment into **microplastics (MPs)**

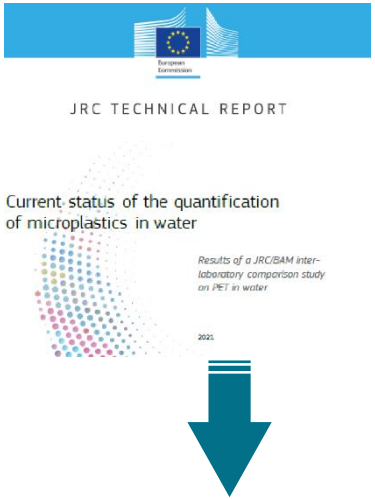
__MPs__ are small solid particles composed of __synthetic polymers__ that are __non-biodegradable__ and __< 5 mm__ in size according to ECHA, or __1 - 1 000 µm__ according to ISO/TR 21960:2020

__The 2020 EU's__ Circular Economy Action Plan (__CEAP__) encourages sustainable consumption and prevention of plastic waste

Current MPs' measurement requirenments:

- **Dinking Water Directive: (DWD 2020/2184) –** requirenment for measuring MPs in drinking water from January 2024
- **Urban Wastewater Treatment Directive (UWWTD, 2024/3019) –** requirement for MPs monitoring
- **Environmental Quality Standards Directive:** under revision (surface water and groundwater)
- **Regulatory framework on contaminants in food:** need for development and harmonisation of analytical methods for measuring MPs in food

Measurement gaps and challenges



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EFSA Scientific Colloquium 25 – A coordinated approach to assess the human health risks of micro- and nanoplastics in food

Results of WEPAL-QUASIMEME/NORMANs first global interlaboratory study on microplastics reveal urgent need for harmonization

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NEED

- *Harmonisation of definitions* **(analyte? relevent measurands?)**
- Overcoming challenges arising from *low levels* and *matrix complexity* **(reliable sample prep/filtration/ pre-concentration?)**
- **RTMs/RMs** for instrument calibration, methods validation and quality control purposes
- Improved comparability amongs methods and laboratories to support environmental monitoring agencies and testing laboratories, policy makers and further research => *harmonisation of methods*

LGC

Addressing metrological needs

Sample Preparation

fractionation
(G-SPLITT and AF4)

filtration

chemical digestion

Detection and Characterisation

size + number concentration
(spICP-MS)

size + number concentration + chemical identification
(LD-IR)

size + number concentration
(DIA)

RTM/RM development

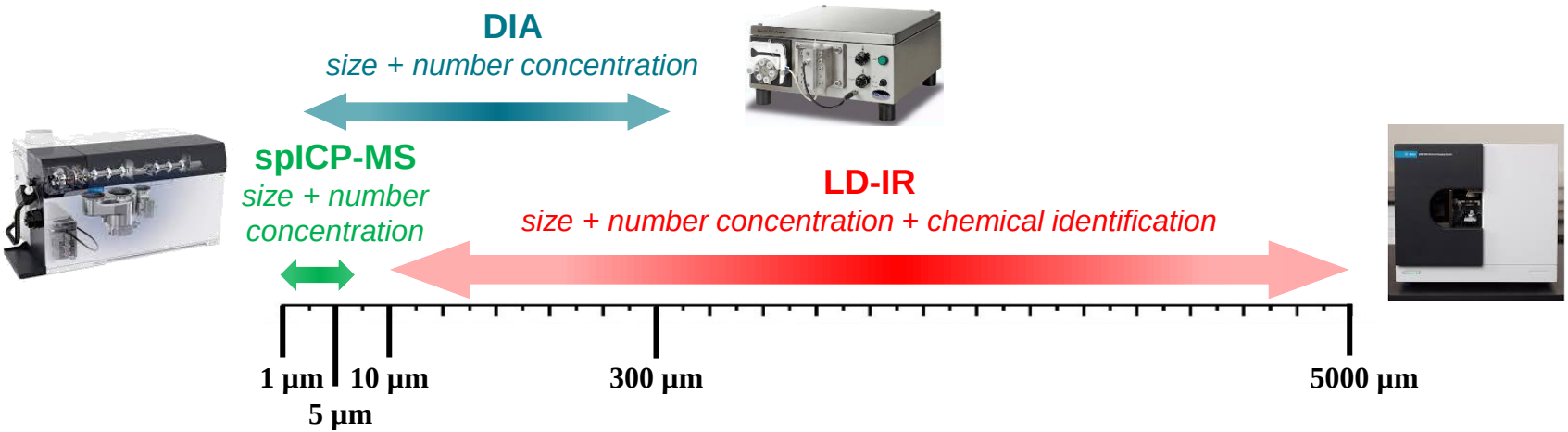
- in suspension
- on solid support

At **NML** we are addressing metrological needs through development of:

- ✓ ***Sample preparation strategies***
- ✓ ***Complementary characterisation methods***
- ✓ ***RTM/RM***

Platform development / *Detection systems*

- ✓ To better understand environmental and health impact arising from microplastics pollution, reliable methods for their detection and quantification are needed
- ✓ NML's ***multimethod characetrisation platform*** covers entire **MPs size range** by using 3 complemetary techniques:
  - single particle Inductively coupled plasma mass spectrometry (**spICP-MS)** / Dynamic Image Analysis (**DIA)** *for small MPs*
  - Laser Direct Infrared **(LD-IR)** /Dynamic Image Analysis (**DIA)** for larger MPs
- ✓ Addresses **key measurands:** *particle size* and *number concentration*
- ✓ Other important measurands: *chemical identification, morphology*



How to ensure reliable MPs characterisation?

I
Determination of instruments' applicable **size** range, and accuracy in size characterisation

Can be verified using commercially available reference materials characterised for particle size

II
Verification of qualitative **polymer identification**

Can be verified (against reference library) using commercially available reference materials of varied sizes and known polymer composition

III
Determination of instruments' applicable **number concentration** range, and accuracy in particle counting

Can be verified using samples with known particle number (solid or suspensions) characterised with orthogonal methods

IV
Evaluation of efficiency of **sample preparation** (*via* filtration)

Can be verified using particle suspension (matrix match) of known particle number concentration (characterised with orthogonal methods)

V
Evaluation of laboratory **blank levels**

Can be verified using ultrapure water (or blank solvent) prepared in the same way as a sample

detection (i.e. instrument/technique specific)

sample prep.

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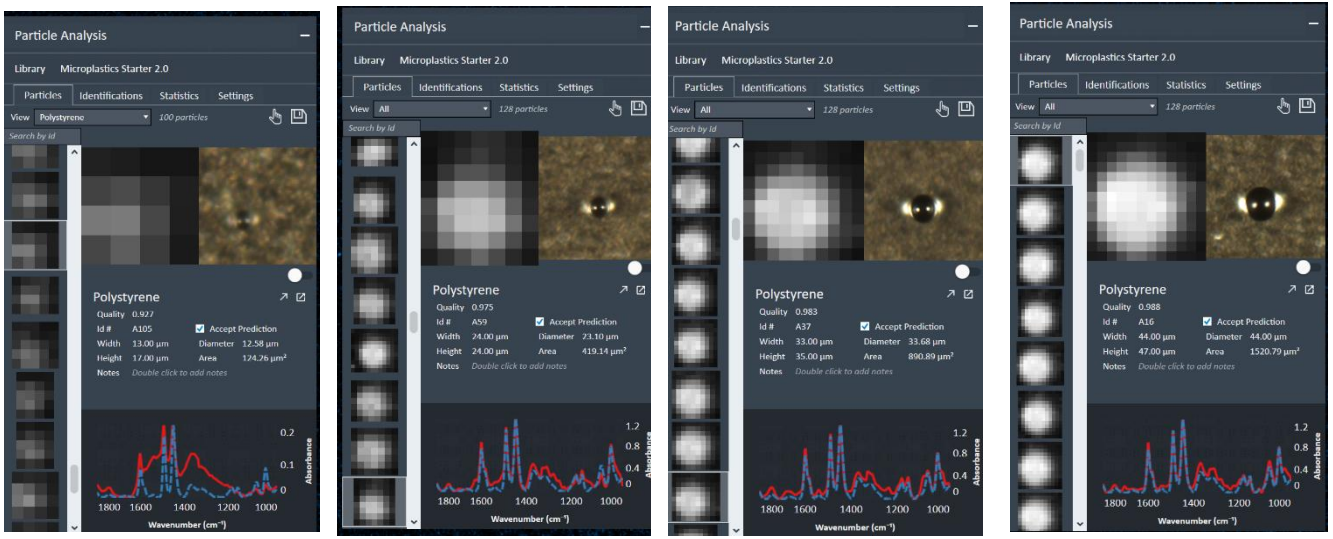
Particle size and chemical identification by LD-IR



Model sample: *PS particles*

10 µm PS Duke (Thermo)

20 µm PS Duke (Thermo)

30 µm PS Duke (Thermo)

40 µm PS Duke (Thermo)

Nb of PS particles detected		Mean Diameter (µm)	StDev	RSD%	Size Recovery *(%)
PS10µm	29	12.6	2.3	18.0	125.3
PS20µm	31	23.4	2.5	10.5	111.7
PS30µm	17	32.2	3.2	10.0	106.6
PS40µm	23	41.9	3.0	7.1	93.8

From particle size provided by manufacturer, characterised by optical microscopy

- All particles identified correctly as PS, across tested size range
- Measured particle size in agreement with size provided by the manufacturer, across tested range
- Larger uncertainties associated with recoveries observed for sizes close to the size LOD of the instrument

**HQI describes how closely the spectrum of the sample matches that in the reference library

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Particle counting

*In the absence of solid samples with known particle number, particle suspensions can be used to evaluate **accuracy in particle counting** but this involves **sample prep via filtration!***

Several filtration systems are available, but in all cases:

It might be difficult to deposit particles in the middle of the filter

Solution:

Typical scanning filter area (~16mm), can be divided into smaller parts then data combined

Deposition area can be reduced:

in-house cut silicon-seal

Particles might dissociate from the filter

Solution:

Reduce electrostatic forces

Ohaus ION-100A
(static ionizer)

Sample preparation *via* filtration

What to consider?

- Characteristics of MPs
- Characteristics of the solvent
- Particle loses upon filtration (e.g. sticking to glassware)
- Particle agglomeration/aggregation (including coffee ring effect) upon filtration

EU directive 2020/2184 ***:*** **“The analysis procedure shall be considered acceptable, if the recovery rate is within the range of 100% to +/- 40%”**

- Recovery rate can be calculated using particle suspension with known particle number concentration by comparing particle number counted with LD-IR vs. particle number present in the filtered suspension
- In the absence of reference materials characterised for particle number concentration, an orthogonal technique (e.g. Optical Particle Analyser) can be used to characterise particle suspension prior to filtration

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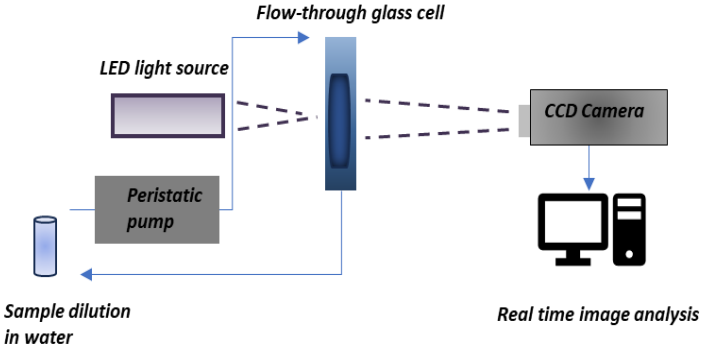
Evaluation of efficiency of **sample preparation** (*via* filtration)

Can be verified using particle suspension (matrix match) of known particle number concentration (characterised with orthogonal methods)

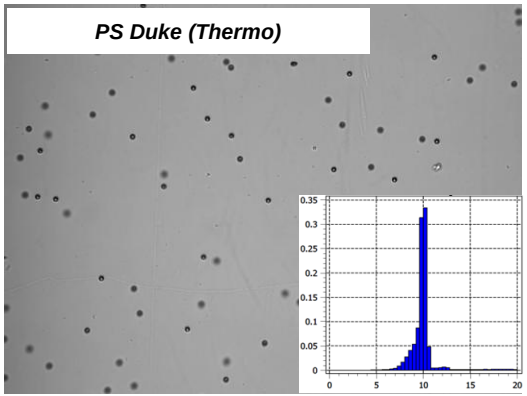
In the absence of Certified Reference Materials (CRMs) characterised for particle number concentration, testing laboratories could use orthogonal methods to assign a value to in-house quality control materials

Orthogonal approach: *Dynamic Image Analysis (DIA)*



Working Principle:

- ✓ particle suspension is pumped through the flow-through cell illuminated by a LED light source
- ✓ high resolution CCD camera (1024 x 768 pixel) takes images continuously, which are analysed in real-time with build-in software



Material	Mean size (µm)	Size recovery (%)	Particle number concentration (kg^{-1})	Number concentration recovery (%)*
PS Duke	10.04 ± 0.10	99.7	(3.76 ± 0.15) E+09*	96.0

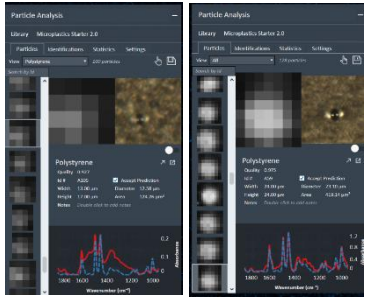
Mean ± standard deviation, n=45

*Particle number conc *theoretically* calculated, assuming bulk density of PS, mass fraction and *size characterised by optical microscopy*

- Particle size measured with DIA in agreement with the size provided by the manufacturer
- Particle number concentration in agreement with value calculated theoretically using size and PS mass fraction

Complementarity between LD-IR and DIA

LD-IR

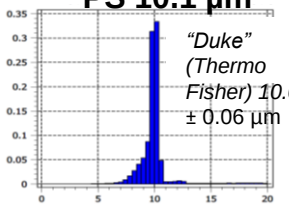


Sample	Particle size (µm)	
	LD-IR (mean ± stdev)*	DIA (mean ± stdev, n=15)
PS 20 µm	24.8 ± 2.5	20.5 ± 4.0
PS 10 µm	12.7 ± 2.6	10.04 ± 0.10

*Mean ± stdev from 100 PS particles deposited on a gold filter

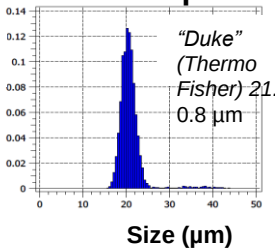
DIA

PS 10.1 µm



"Duke" (Thermo Fisher) 10.07 ± 0.06 µm

PS 21 µm

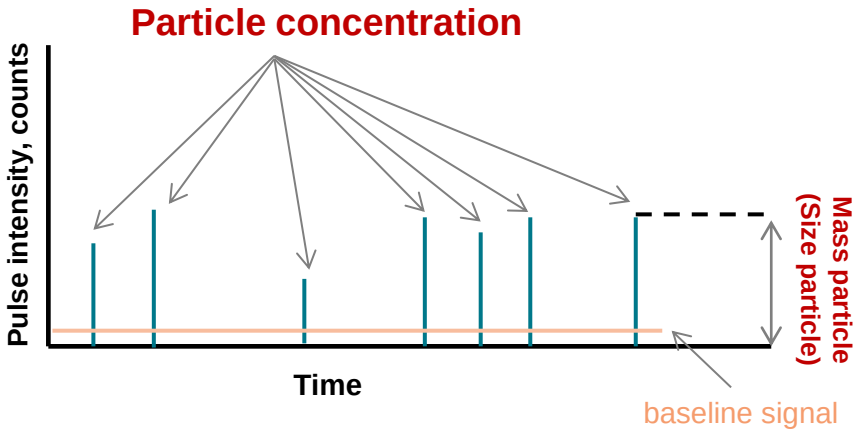


"Duke" (Thermo Fisher) 21.0 ± 0.8 µm

LD-IR detected and identified as PS 100% of deposited particles

Agreement between DIA and LD-IR for MPs size determination achieved

Orthogonal approach: single particle (sp) ICP-MS



Working Principle:

- ✓ Each transient peak or pulse represents a single particle event – number of peaks represents **particle number concentration**
- ✓ Signal intensity represents mass of element per particle, which for known geometry and chemical composition can be translated to **particle size**
- ✓ From baseline signal information about the dissolved ions
- ✓ Detection of MPs through ^{12}C or ^{13}C

Complementarity between (sp)ICP-MS and DIA

spICP-MS

Technique	PSL 5 µm Particle number concentration (g^{-1})	
	Mean (g^{-1})	Expanded uncertainty, *k=2* (g^{-1}) (REU%)
spICP-MS (n=45)	8.38 E+05	1.99 E+05 (23.7%)
(DIA) (n=45)	8.24 E+05	4.24 E+04 (5.1 %)

REU% Relative expanded uncertainty
Polystyrene Latex (PSL)

DIA

Agreement between DIA and spICP-MS for MPs number concentration results obtained

How to ensure reliable MPs characterisation?

V

Evaluation of laboratory **blank levels**

Can be verified using ultrapure water (or blank solvent) prepared in the same way as a sample

Laboratory blank levels

Laboratory blank levels can significantly influence particle number measured in the sample and should be evaluated!

Keeping blanks under control:

- Laboratory floors made of washable material and cleaned frequently
- Laminar flow cabinet at least ISO Class 5 (3520 particles/m^3)
- Cotton or antistatic lab coats

Evaluation and reporting:

- Blanks (ultrapure or pyrogenic water) should be evaluated within each sample batch, prepared using the same procedure as samples
- Reported results (on samples) should be presented without prior subtraction of blank values
- Blanks should be reported along samples

Determination of the Limit of particle detection (LOD) and quantification (LOQ)

$$\textbf{LOD} = (\text{mean}_{\text{n blanks}} + 3 \times \text{StDev}_{\text{n blanks}})$$

$$\textbf{LOQ} = (\text{mean}_{\text{n blanks}} + 10 \times \text{StDev}_{\text{n blanks}})$$

Priority Polymers according to EU Directive: ***Methodology to measure microplastics in water intended for human consumption*****:** PS, PP, PE, PET, PVC, PA, PU, PTFE, PC, PMMA.

Summary

- LD-IR is a promising technique allowing determination of MPs size (and morphology) and chemical composition
- Steps required to achieve reliable particle characterisation with LD-IR have been proposed and discussed
- Performance of LD-IR have been evaluated using model MPs composed of PS in size ranging from 10-40 µm achieving good agreement with values determined using orthogonal approaches
- Complementarity of DIA/spICP-MS has been investigated using PS microparticles and good agreement obtained

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