SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

Analisi di Microplastiche

Analisi per forma (LDIR) e per massa (Py-GCMS)

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What are Microplastics?

- Microplastics are defined as polymer particles **<5mm**. They are now recognized as an **emerging contaminant** in the environment, however current analytical methods for their **identification** and **quantification** are not fully developed.
- Of most concern are the particles **<50µm** as they are the ones that are most likely to be **end up in our food chain.**

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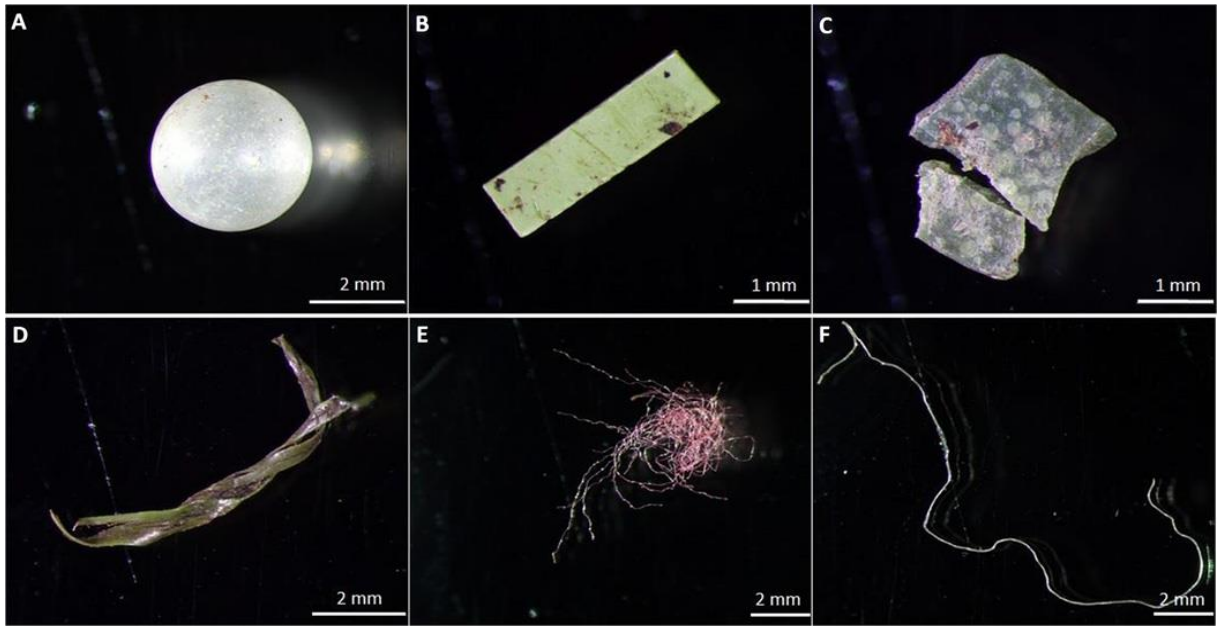
Measurement Challenge

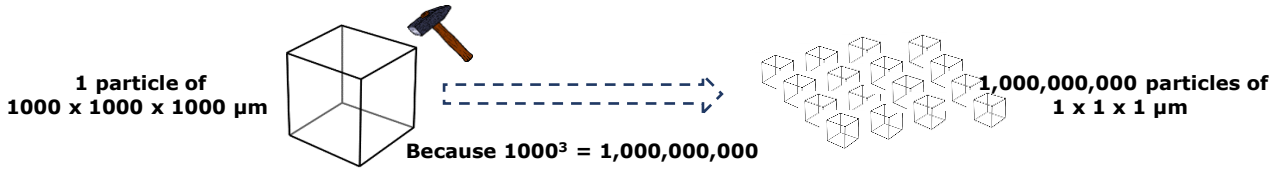
- Microplastics are difficult to measure, not only due to their obviously small size, but also because the chosen technique needs to be able to identify a wide variety of polymers existing in a wide variety of different shapes and forms e.g. fibres, fragments and microbeads.
- **<u>There is no single “perfect” technique, so a combination of approaches is needed.</u>**



Measurement Challenge

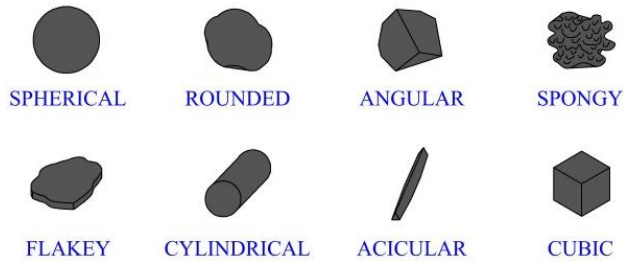
Microplastics finds have been reported as number of particles

- Particle counts are not a conserved base quantity – particles are brittle



Different size ranges are used (e.g. 330-5000 µm versus 10-500 µm)

Particle shape is ignored – size is reported by one number only, typically called the “particle size”



All these particles would be treated as having the same size

Particle numbers + sizes are important as impacts increase with decreasing size

BUT

The **mass** of microplastics is needed to assess and compare sources and contamination levels

Measurement Challenge

Forma

Spettroscopia LDIR

- Dimensioni delle particelle
- Distribuzione morfologica
- Forma (fibre, sferiche, cilindriche,…)
- UDM: **n° di particelle/ kg o L di campione**

Massa

Pirolisi GCMS

- “Quanta massa di microplastiche ho”
- Analisi quantitative (MP e additivi, std interno o esterno)
- UDM: ug/ kg o L di campione

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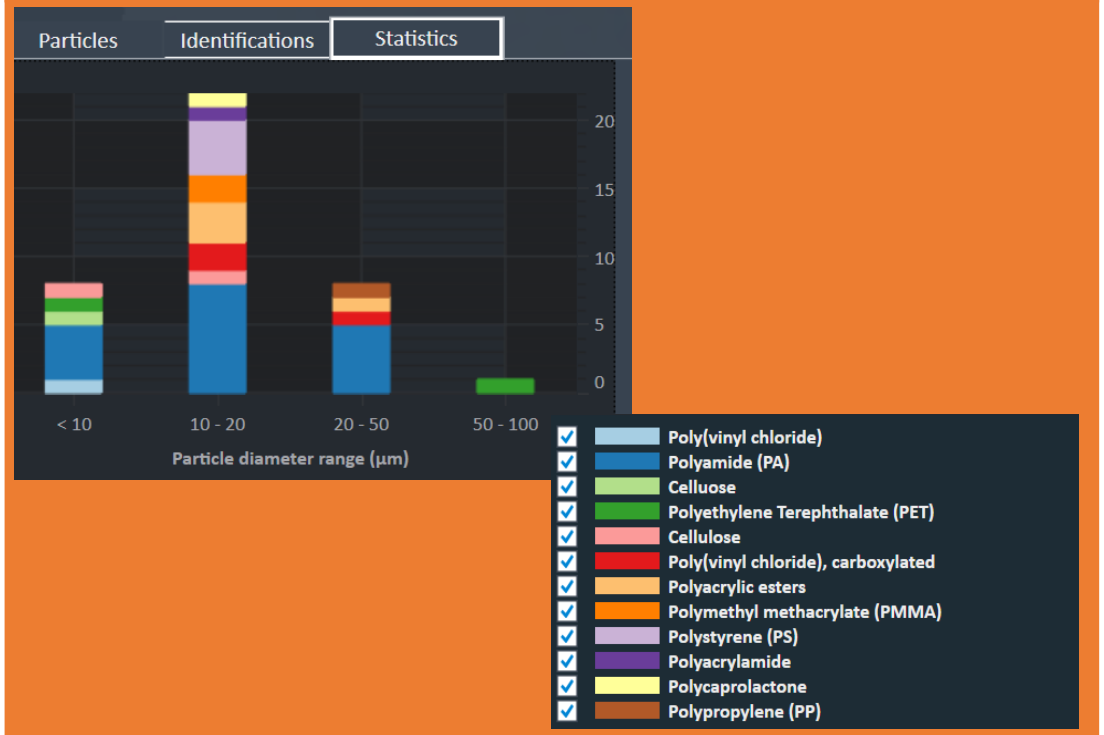
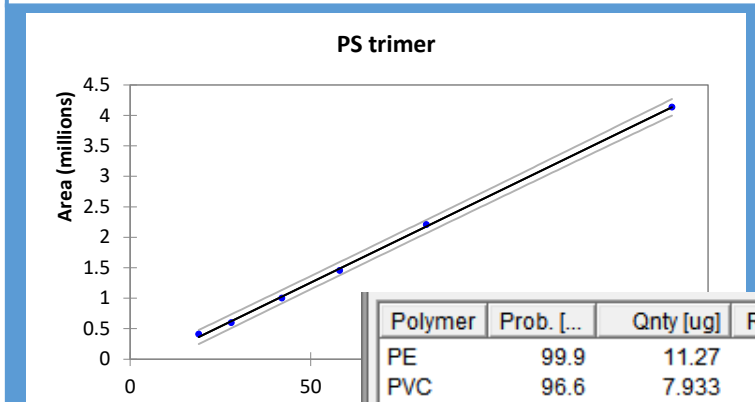
Measurement Challenge

Forma



Massa



Polymer	Prob. [...	Qnty [ug]	Ratio [%]	Area	RT [min]
PE	99.9	11.27	49.3	31420	16.36
PVC	96.6	7.933	34.7	146285	10.57
SBR	23.9	0.855	3.74	12294	7.07
PP	92.1	0.724	3.17	4116	6.46
PS	99.6	0.577	2.53	75144	21.33
PET	88.6	0.490	2.14	2574	13.92
PMMA	100.0	0.367	1.61	39050	4.82
PU	99.9	0.305	1.34	81556	18.01
ABS	70.5	0.152	0.67	2697	18.02
N66	88.0	0.111	0.48	4847	6.62
N6	81.3	0.061	0.27	3745	11.50
PC	70.8	0.018	0.08	5019	11.24
			(100)		

Measurement Challenge

Forma

Massa

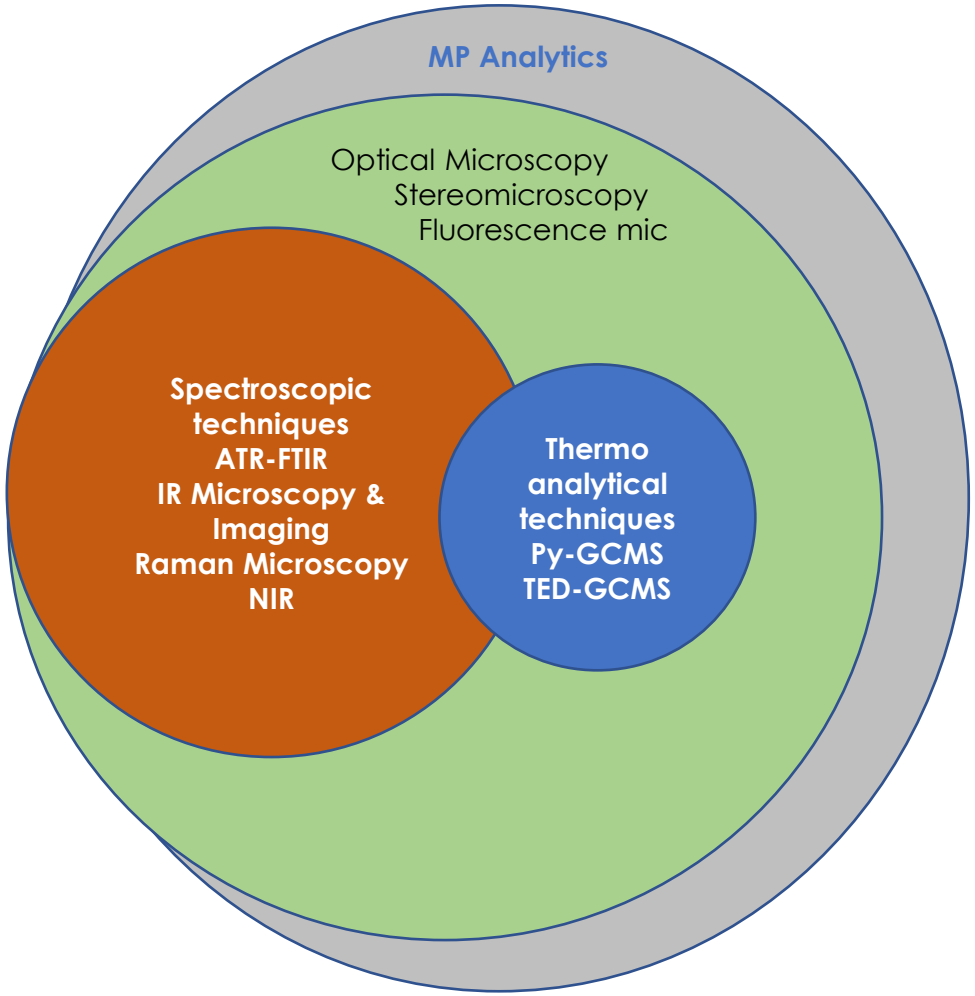
Analisi per forma: LDIR

Forma

Spettroscopia LDIR

- Dimensioni delle particelle
- Distribuzione morfologica
- Forma (fibre, sferiche, cilindriche,…)
- UDM: **n° di particelle/ kg o L di campione**

Analisi per forma: LDIR



Analytical technique	Shape info	Chemical ID	MP_{Num}	MP_{Mass}
Optical microscopy; Fluorescence microscopy	● ●	● ●	● ●	● ●
ATR-FTIR	N/A	●	N/A	● *
µFT-IR-Imaging	●	●	●	◐
NIR, Hy-Spec-Imaging	●	●	●	●
µRaman;	●	●	●	●
Pyr-GC-MS	●	●	●	●
TED-GC-MS	●	●	●	●

*** Using a balance to weigh particles**

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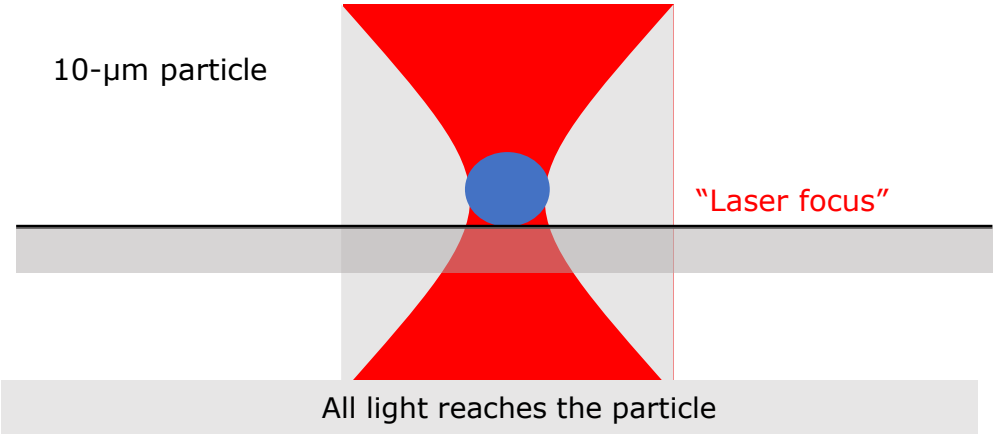
Laser Direct Infrared (LDIR) Spectroscopy

- New instrument architecture
- Bright infrared laser source with high power density (1000x more than a Globar)
- Proprietary Agilent quantum cascade laser (QCL) technology
- Rapidly tunable across the mid-infrared (1800 – 975cm^{-1})
- Laser spot size at sample is 5.5 – 10µm diameter

- Focus all laser power onto a particle
- One second per spectrum
- 0.5cm^{-1} spectral resolution

Coherent laser light

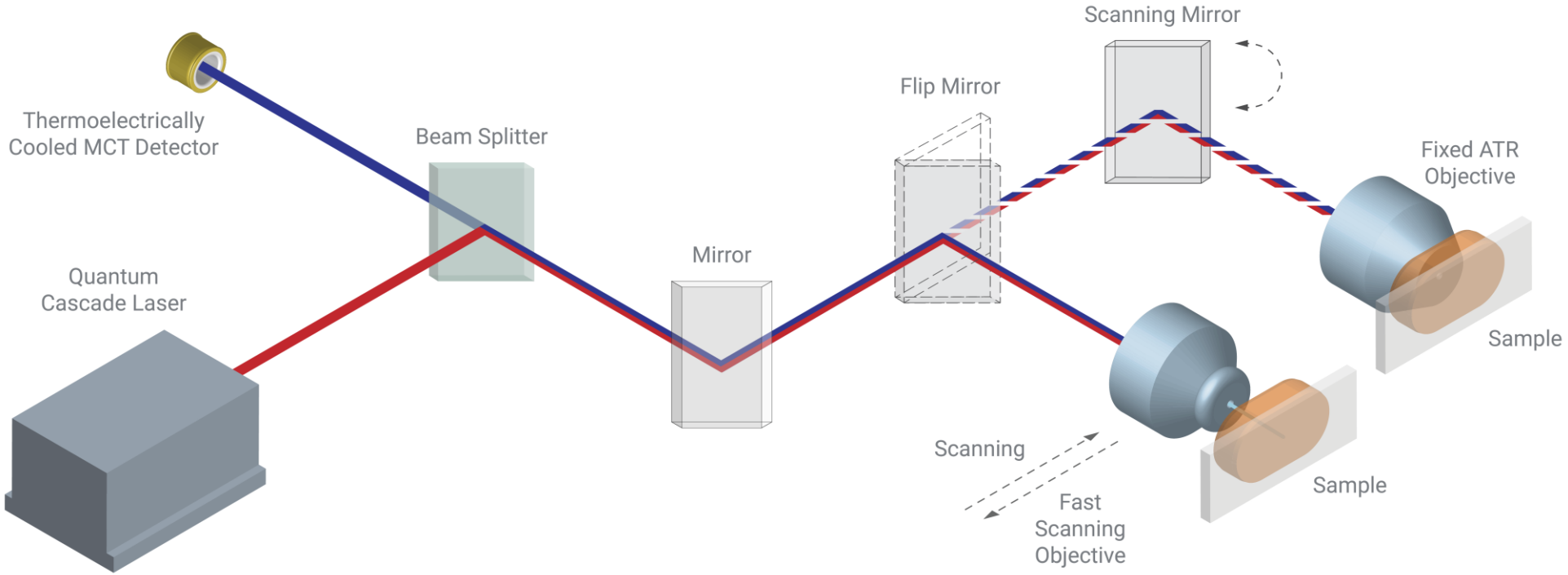


LDIR 8700- QCL Setup

The LDIR is NOT an FTIR. It doesn't have an interferometer.

The laser wavelength is selected using a monochromator (same type as we use in the Cary 3500) so it's a form of "dispersive IR" spectrometer.

MCT detector doesn't use LN2 – it's electrically cooled – so always ready to use

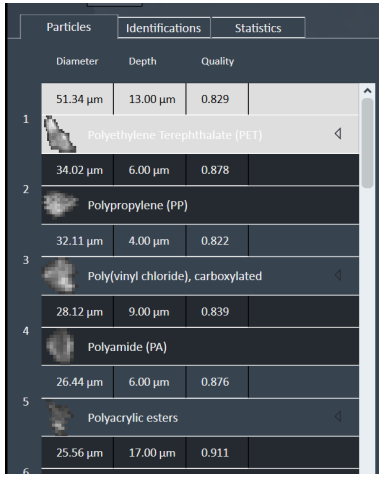


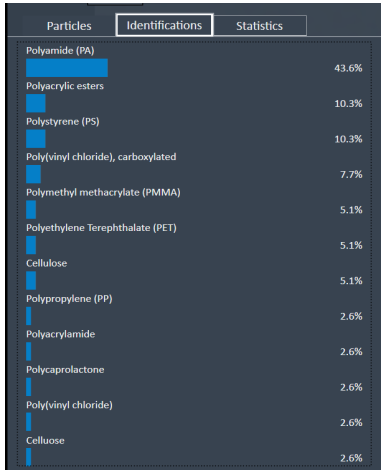
LDIR Microplastics Analysis Workflow

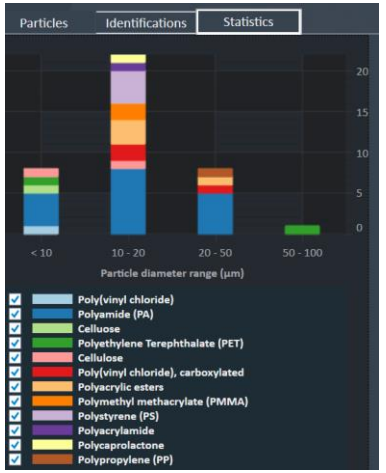
Locate and measure all particles

- Acquire spectrum
- Compare to standard IR library
- Update statistics

For every particle







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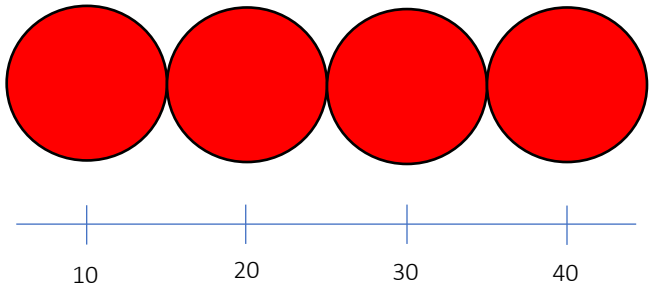
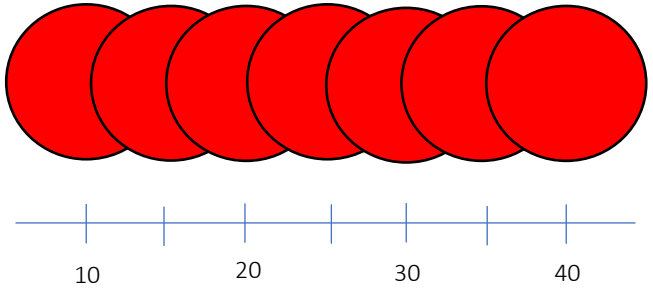
How do we achieve such great resolution?

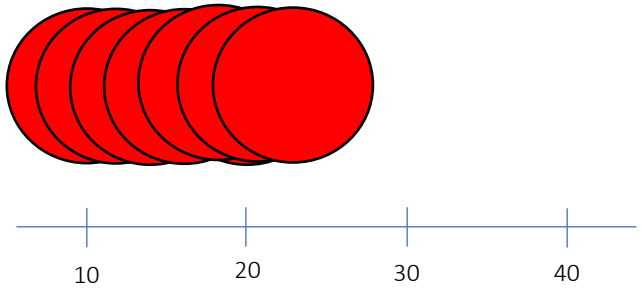
Oversampling



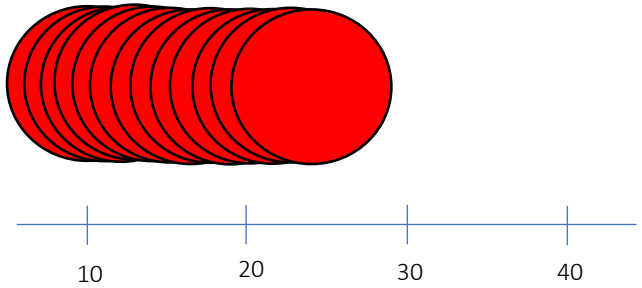
10µm Pixel Resolution



5µm Pixel Resolution



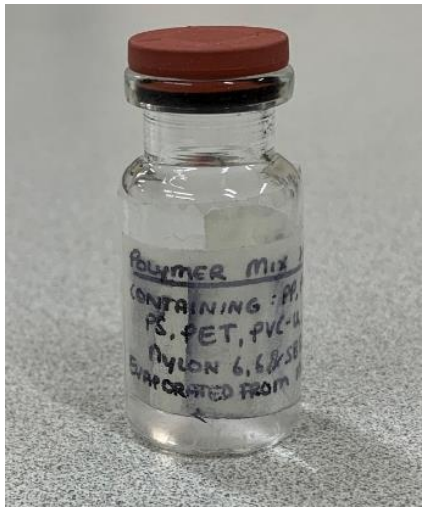
3µm Pixel Resolution



1µm Pixel Resolution

LDIR Analysis of Known Microplastics

- Polymer mix
  - Blend of nine common microplastics ground using a cryomill and filtered to be between 10-100 µm
    - Polyethylene terephthalate (PET)
    - Polyethylene (PE)
    - Polyvinyl chloride (PVC)
    - Polypropylene (PP)
    - Polystyrene (PS)
    - Polytetrafluoroethylene (PTFE)
    - Polymethyl methacrylate (PMMA)
    - Nylon 6,6 (PA)
    - Styrene butadiene rubber (SBR)
  - Particles were suspended in ethanol, sonicated, and aliquoted onto a slide for analysis.

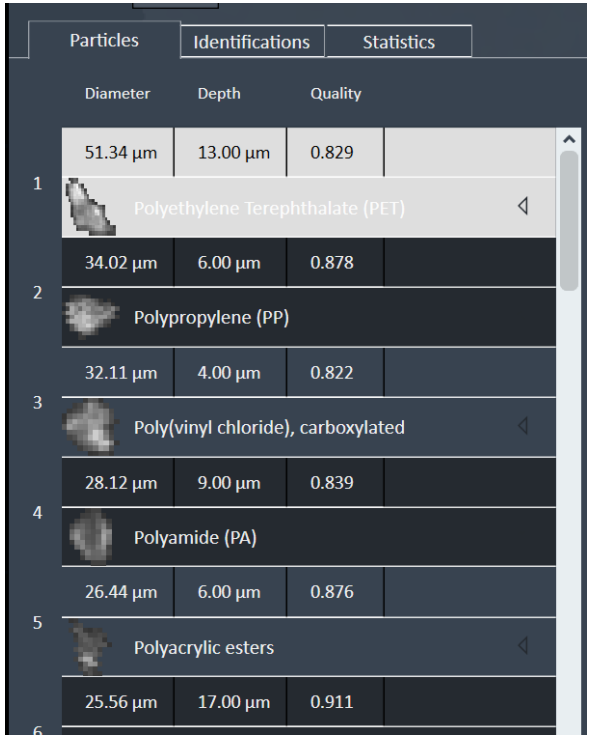


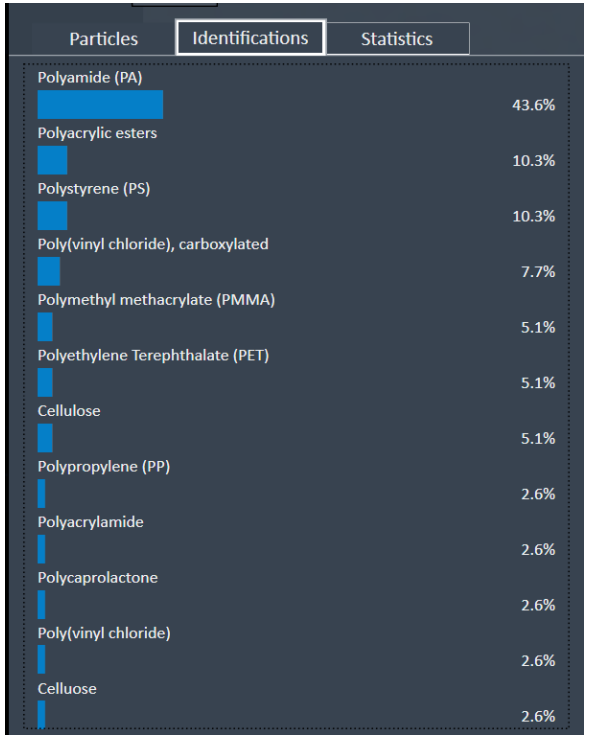
Samples courtesy of
Nikki van Alst / Prof. Jes Vollertsen
Aalborg University, Denmark

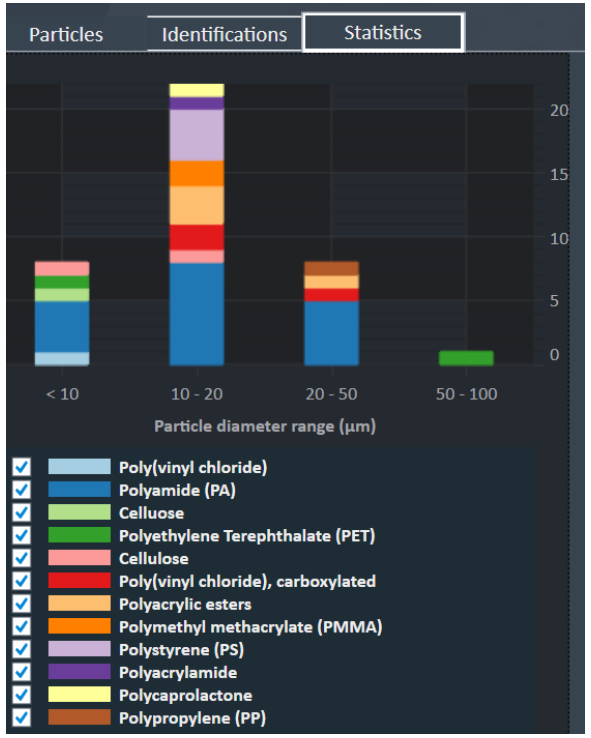
The LDIR can handle a wider range of particle sizes – up to 1000um (1mm) - in a single analysis, so less prep is needed and faster overall measurement time.

Reported Results

Particle breakdown and statistical analysis

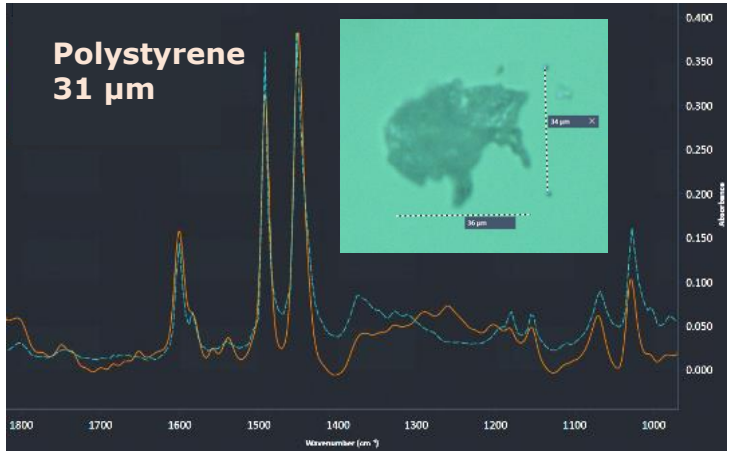


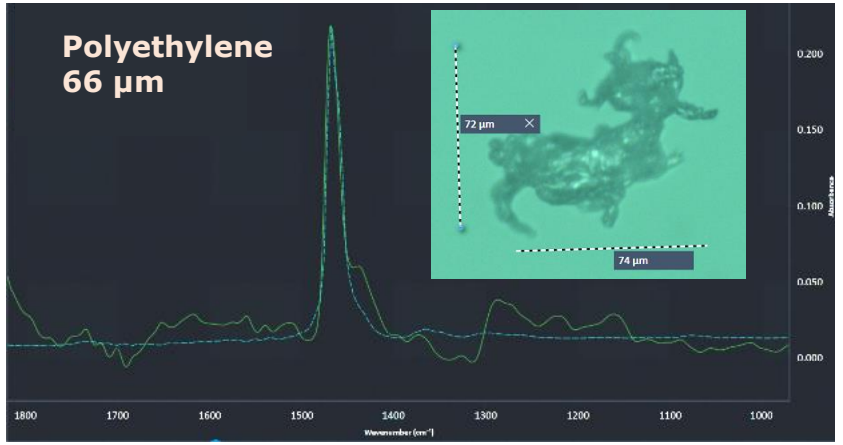


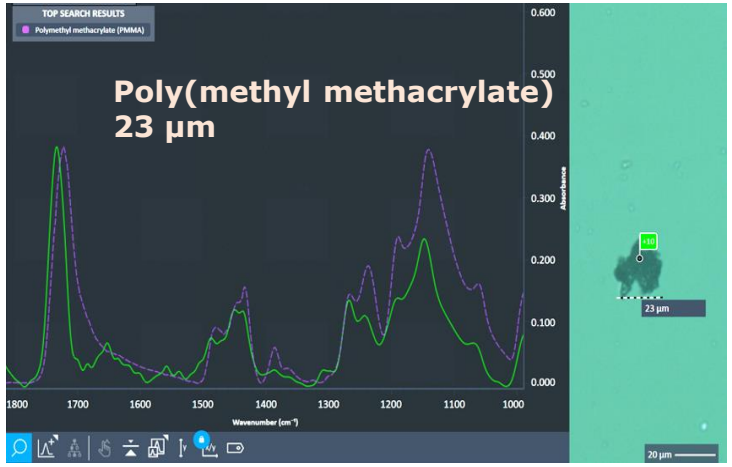


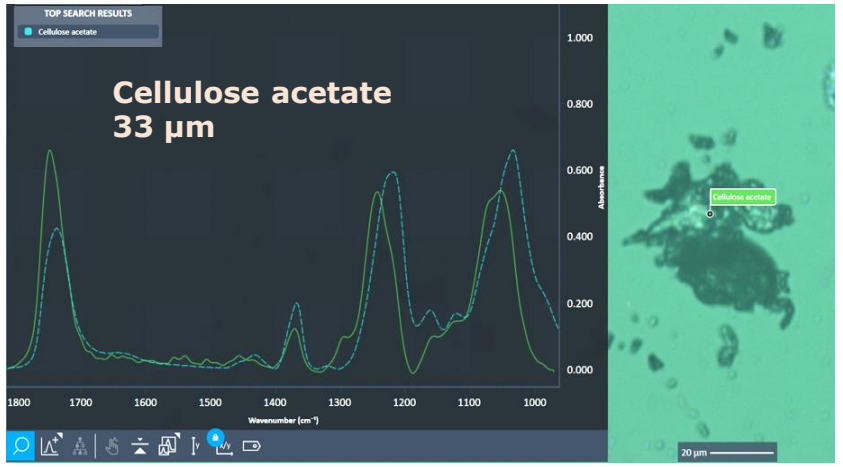
3 mm × 3 mm; 39 particles; 5 minutes

Small Particles

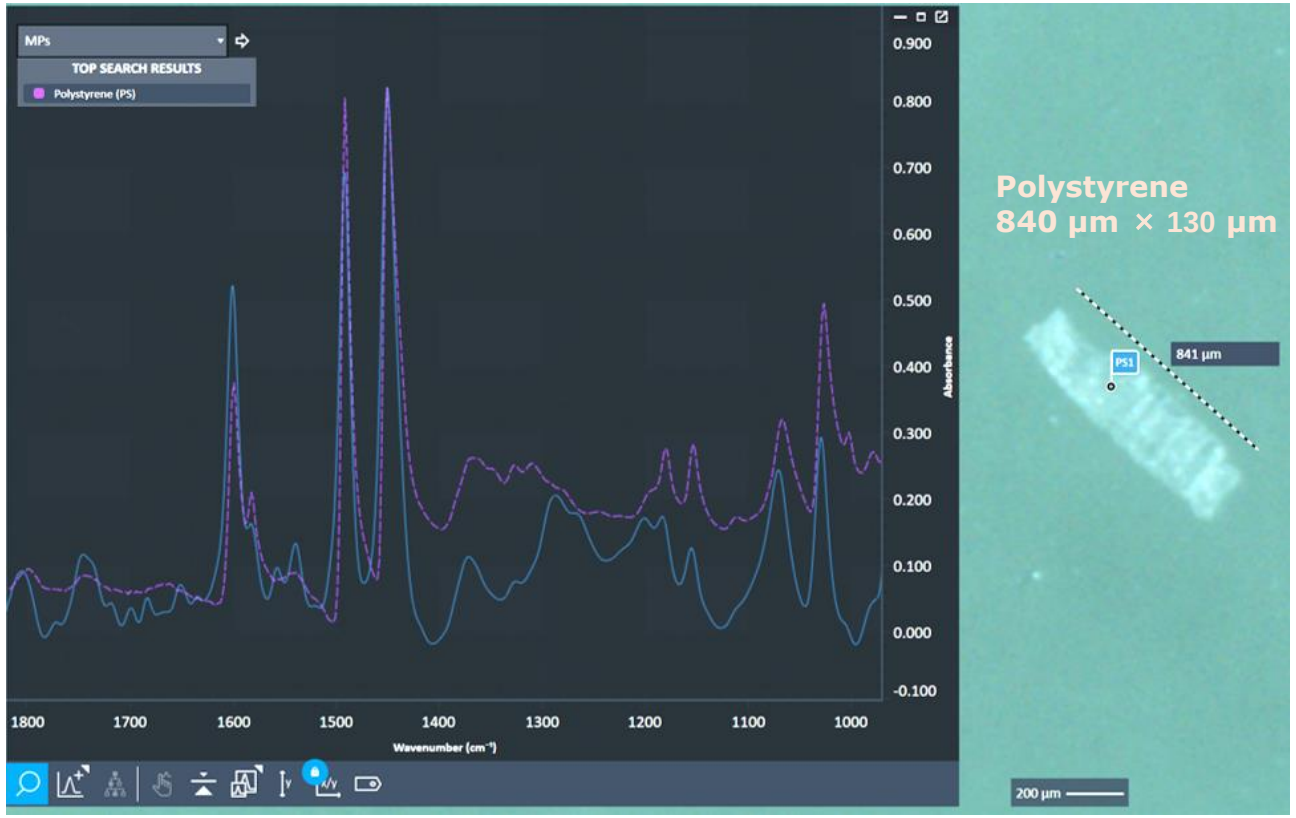








Large Particles



- Both large (1 mm) and small (10 µm) particles can be measured in the same analysis
- Bright laser light is able to transmit through thick particles and ID them

Analisi per massa: Py-GCMS

Massa

Development of analytical method for MPs

Develop an analytical method and analytical system for microplastics analysis using Py-GC/MS

Challenges:

1. Target plastic types
2. Secondary reactions among pyrolyzates (acid, alcohol, amine and unsaturated compounds)
3. Optimization of a separation column for various pyrolyzates
4. Qualification and quantification of plastics (Search software)
5. Matrices difficulties with environmental samples

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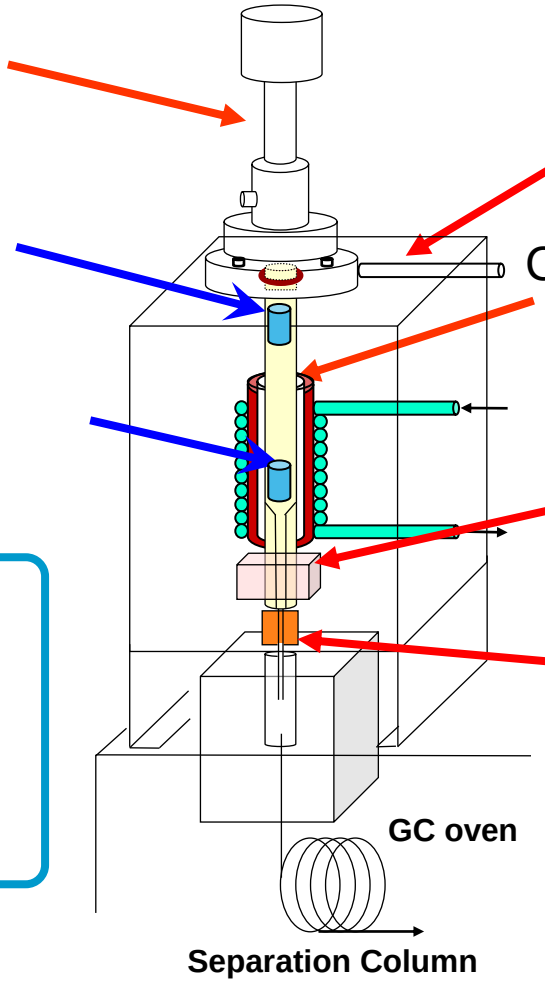
Schematic diagram of Multi-Shot pyrolyzer EGA/PY-3030D

Sampler device

Sample cup waiting position (< 40 ℃)

Sample cup Pyrolysis position



Carrier gas flow path

Ceramic heater

- **Pyrolysis temperature from ambient to 1050C**
- **accuracy 0.1 C**

Interface heater

GC/PY interface union

- **Detection of high-boiling pyrolysates over 100 carbon number**

Sample cup

- **Stainless steel with deactivated coating**
- **Put liquid and solid samples**

Volume: 50µL 80µL

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Target areas

1. **Environmental**
   - MPs in the sea, rivers, lakes, soil, sediments
     - ✓ Waste plastics, Coated fertilizers, Tire wear particles etc.
   - MPs in organisms
     - ✓ Planktons, Fish, Waterfowl etc.
   - MPS in atmosphere
     - ✓ Tire wear particles etc.
2. **Food/drink**
   - MPs in drinks
     - ✓ Water, Beer, Juice etc.
   - MPs in food
     - ✓ Salt, Seafood etc.
3. **Others**
   - Persistent organic pollutants (POPs) on/in MPs
   - MPs from packaging materials in food
   - MPs from Paint and coating materials in rivers

F-Search MPs 2.0

FRONTIER LABORATORIES LTD.

Ver. 1.0

General product information

F-Search MPs 2.0 is a searching software that assists in the **qualitative** and **quantitative** analysis of microplastics by Pyrolysis(Py-)GC/MS method using Frontier's recommended analytical system and analytical conditions.

We developed an analytical method and configured a recommended Py-GC/MS system for microplastics analysis, so it is easy to start microplastics analysis even for those who are using Py-GC/MS for the first time.

Product features

* Japan patent: #6683335

1) Identify Microplastics polymers by a sophisticated search algorithm*

2) Automated creation of calibration curves and quick quantification

Create calibration curves of the registered polymers (ISTD or ESTD) automatically based on the analytical results from the reference polymer mixture. Then, F-Search MPs 2.0 automatically performs quantification, and the results are instantly reported for the environmental MPs.

3) Cover twelve commonly used polymers

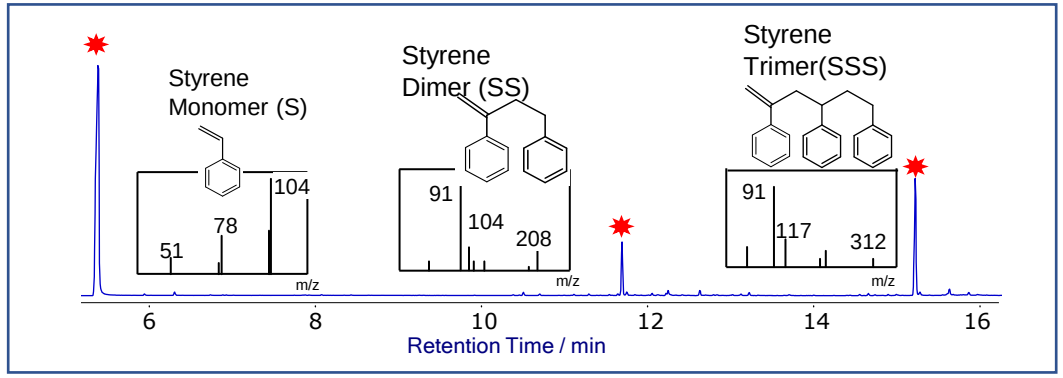
It enables to analyze twelve commonly used polymers (e.g. polyethylene, polypropylene)

4) Enable to create user's own library

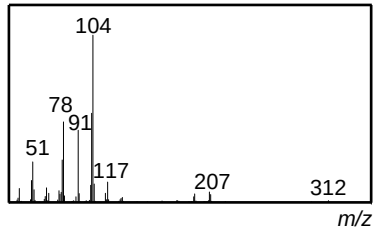
It has a feature to establish user's own library depending on the user's interest.

Comparison of database and searching algorithm

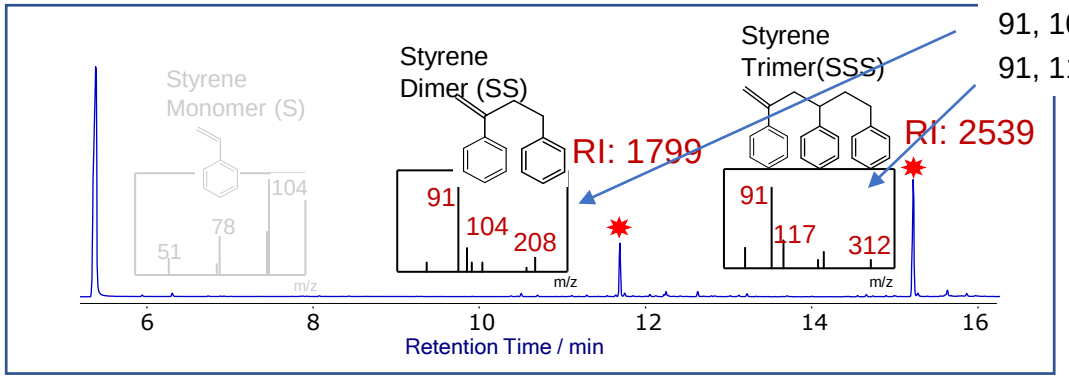
F-Search ver. 3.6 Styrene monomer, dimer, and trimer are selected as the characteristic pyrolyzates, and the mass spectrum obtained by Int-Sum for PS is registered in the database library. For qualification, detect peaks with a certain intensity, create Int-Sum mass spectrum for the sample, and search it on the database library.



Int-Sum Mass spectrum of characteristic pyrolyzates of PS

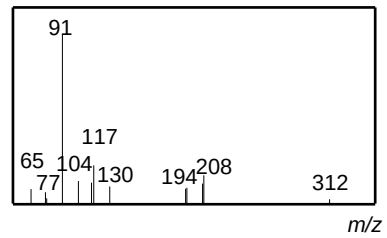


F-Search MPs 2.0 Styrene monomer is not included as a characteristic pyrolyzate because it can be derived from other styrenic polymer, so the Int-Sum mass spectrum of characteristic ions of dimer and trimer is registered in the database. For qualification, these two peaks and characteristic ions are automatically detected from RI and search it on the database library. A styrene trimer is used for quantification.



Characteristic ion
91, 104, 208,
91, 117, 312, *etc.*

Selected ion/peak
Int-Sum Mass spectrum of characteristic ion's pyrolyzates of PS



Search algorithm of F-Search MPs 2.0

A: Registered polymer in database library (e.g. PS)

Database library

Styrene Dimer (SS)

Styrene Trimer(SSS)

RI: 2488

RI: 1749

91 104 208

91 117 312

m/z

TICC

10 12 14 16 [min]

Int-Sum mass spectrum of SS and SSS

91 65 77 104 117 130 194 208 312

m/z

B: Unkown sample (Search for PS)

TICC

10 12 14 16 [min]

Search range

EIC

m/z: 91

m/z: 104

m/z: 208

m/z: 91

m/z: 117

m/z: 312

91 104 208 A

91 117 312 B

m/z

91 65 77 104 117 130 194 208 312

m/z

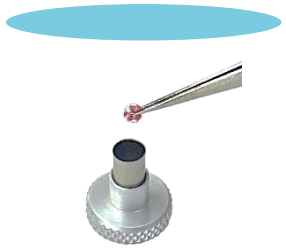
Step 1 :
Detect EIC of characteristic ions of pyrolyzates

Step 2:
Obtain mass spectrum based on Step1

Step 3:
Sum up mass spectrum of A and B

Step 4:
Calculate matching % of mass spectrum between unknown sample and registered polymer in database library

Analytical procedure



Sample Prep

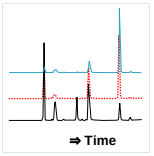
Pyrolysis

F-Search MPs 2.0

Data analysis

Introduction

GC/MS



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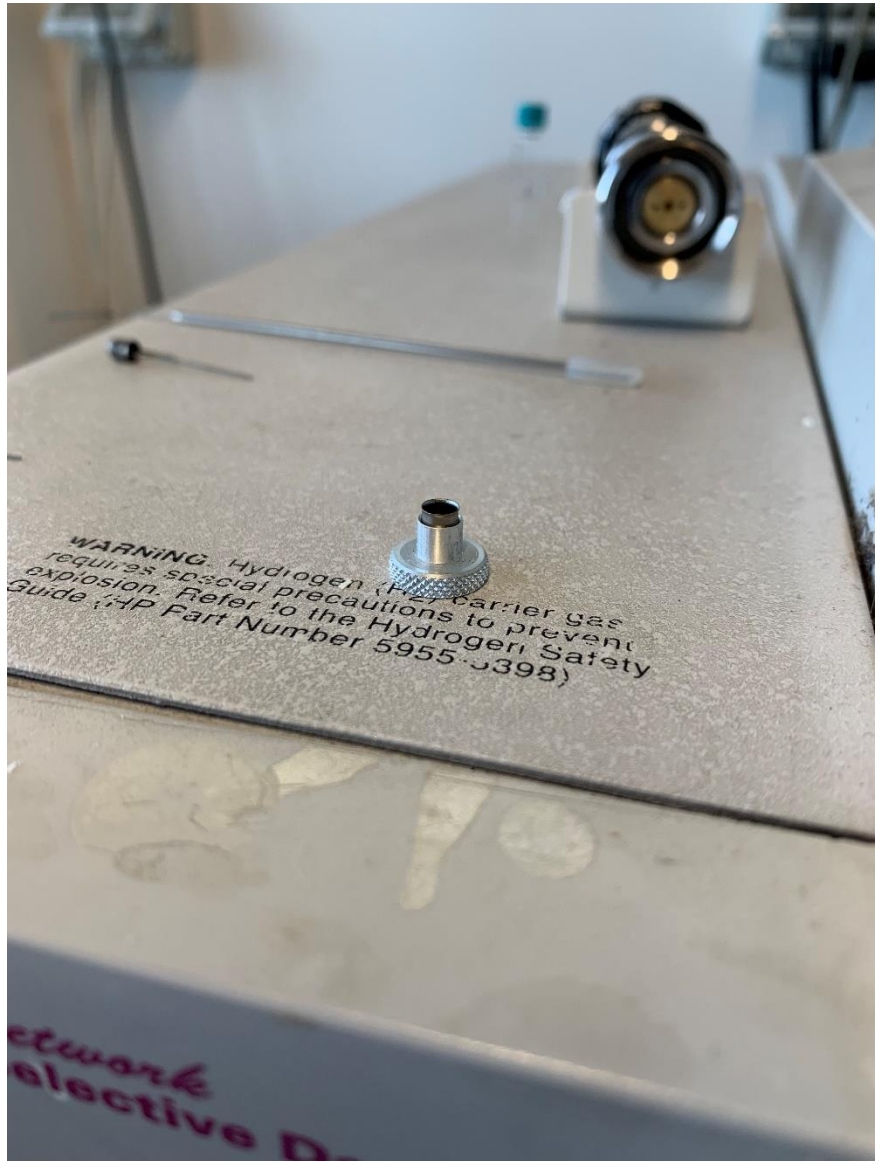

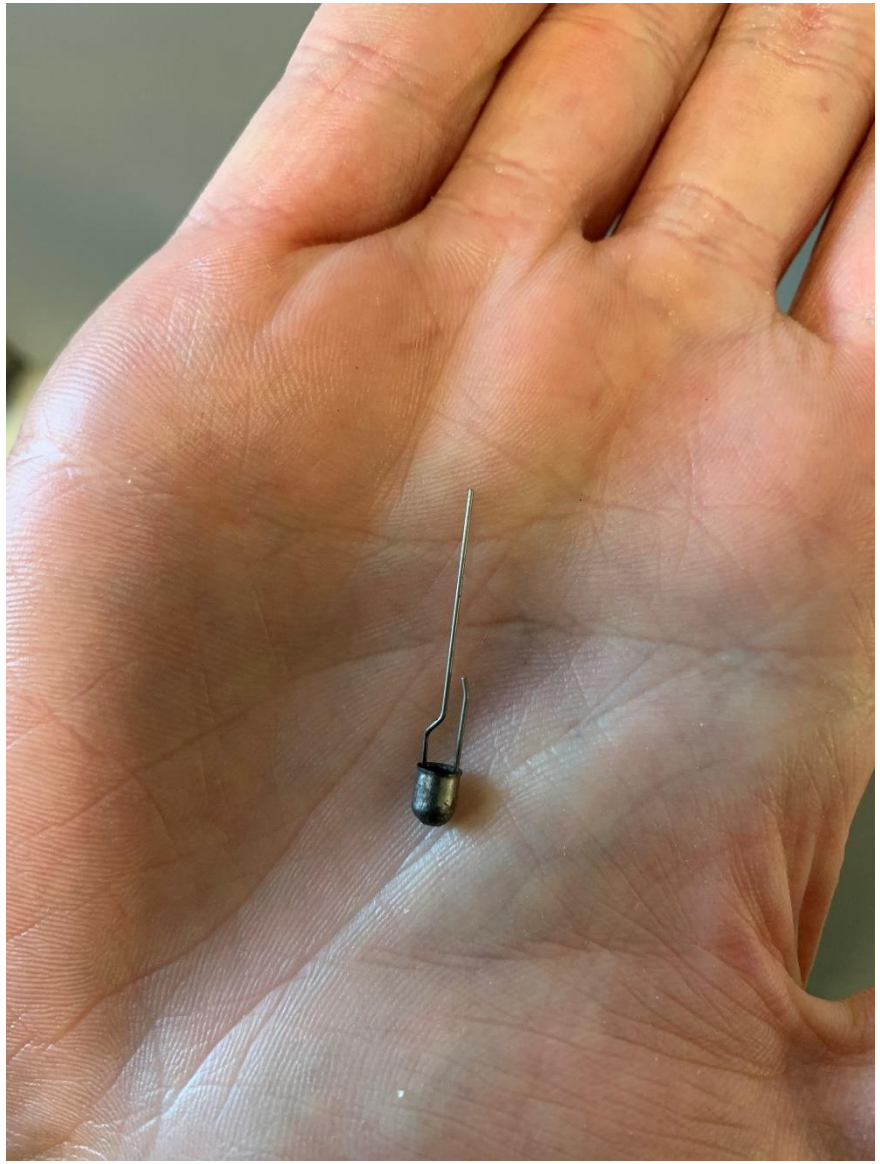

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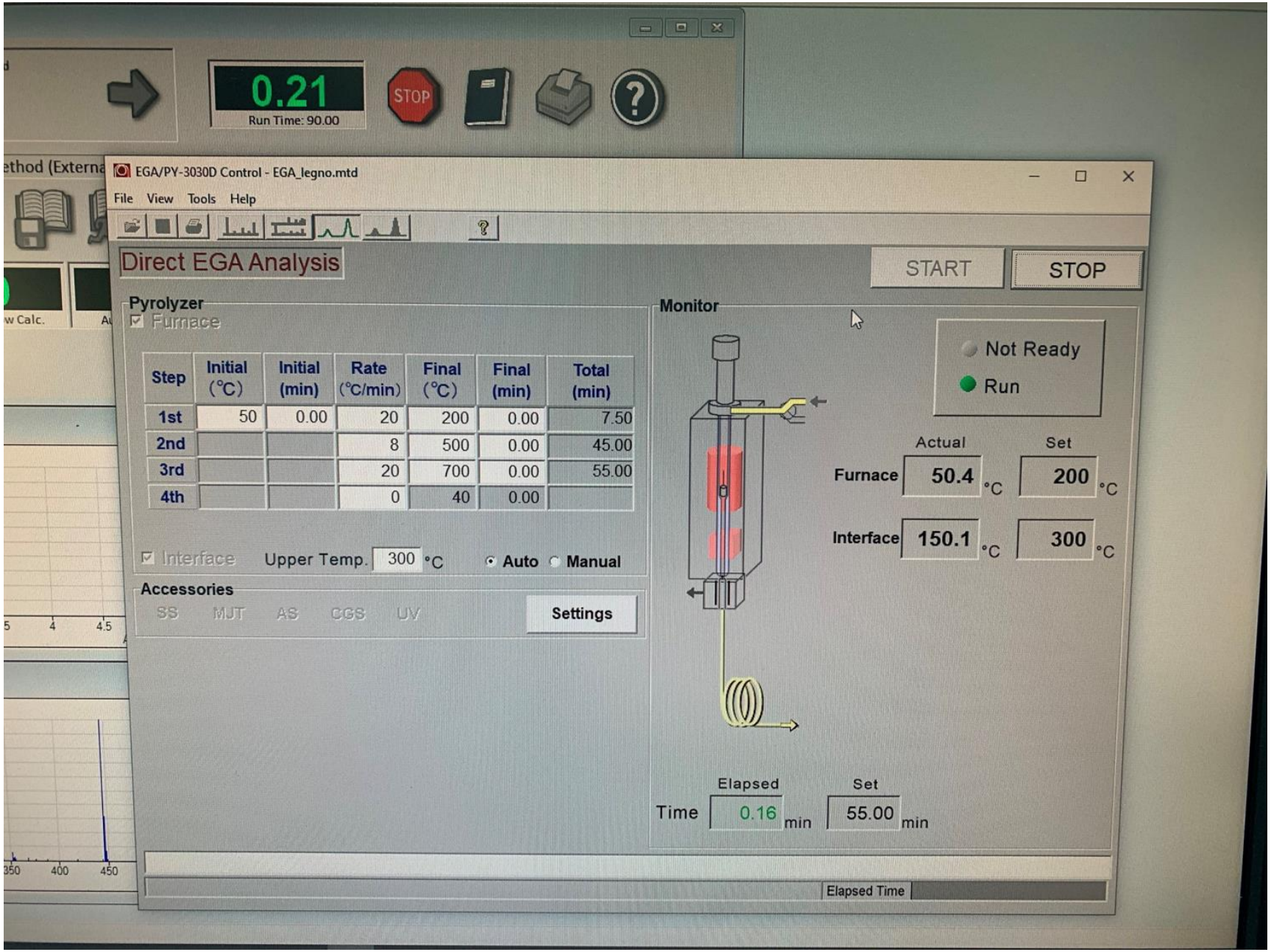

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Sample analysis requires a various fases procedure in order to obtain relevant and consistent results

<u>THIS IS INDIPENDENT FROM THE INSTRUMENT CHOSEN FOR MPs ANALYSIS</u>

Omogeneizzazione **Digestione** **Flocculazione** **Filtrazione**

I vari step di pretrattamento del campione si possono semplificare o rimuovere a seconda della tipologia di matrice che andiamo ad analizzare

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Operational process by F-Search MPs 2.0

Step 1 **Creating a plot of retention index (RI) and retention time (RT)**

RI is created based on the pyrolyzates of Polystyrene using MPs Calibration Standard

Step 2 **Creating a calibration file (QFL)（e.g. 3 points, *n*= 5）**

Calibration file is created based on pyrograms of MPs Calibration Standard

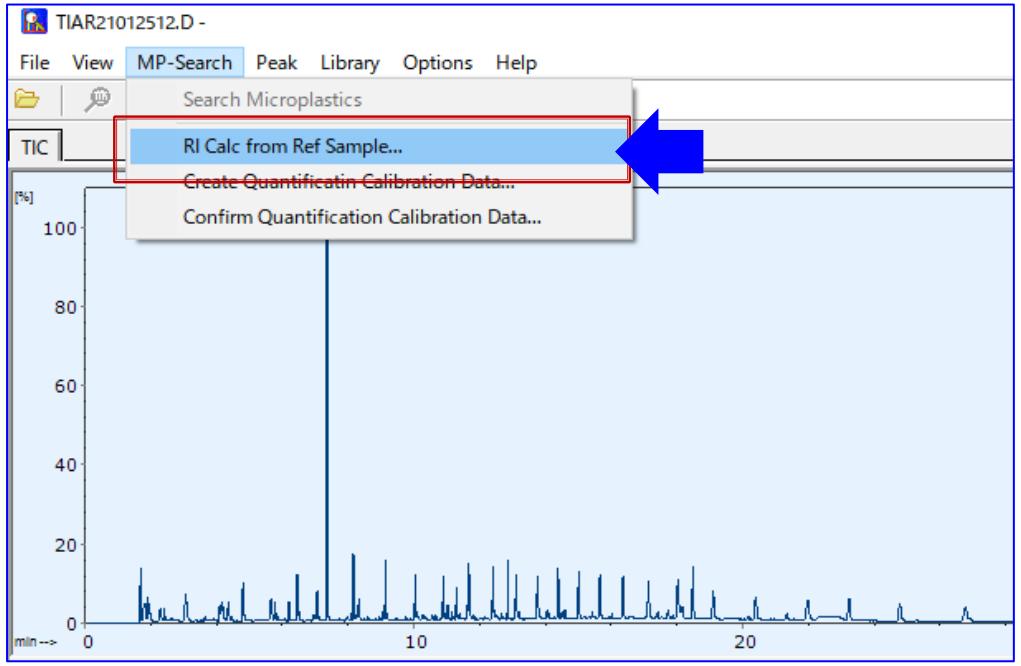
Step 3 **Qualitative/quantitative analysis of MPs**

Search results are displayed

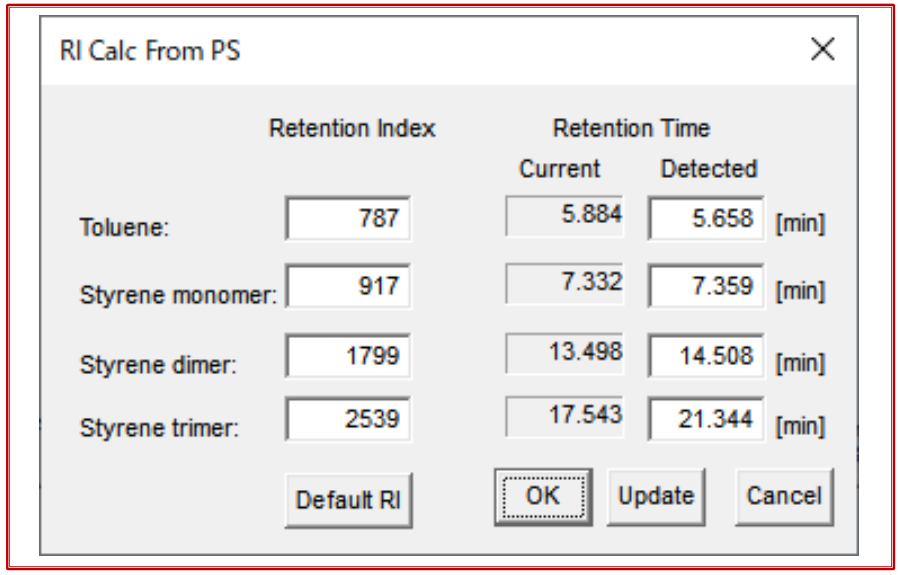
Step 4 **Interpretation of results**

Creating a plot of retention index (RI)

- **RI is created based on the pyrolyzates of Polystyrene in MP Calibration Standard**



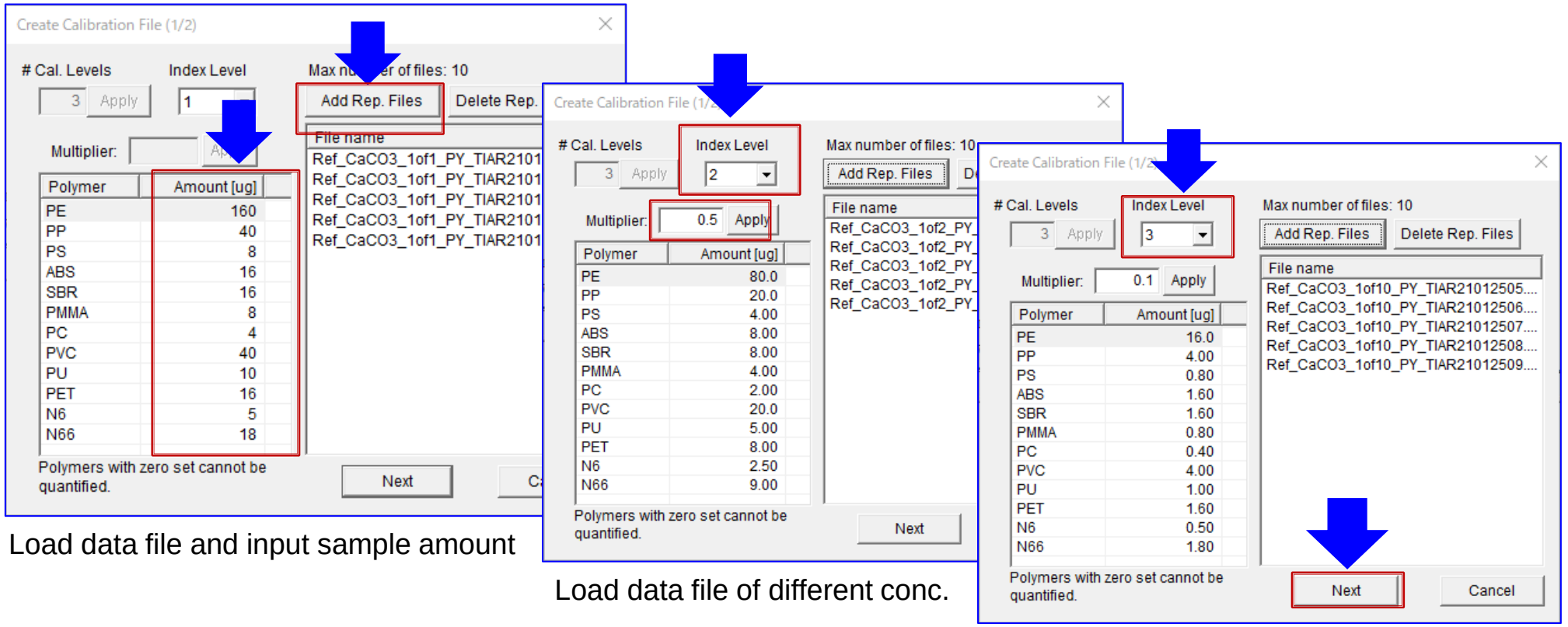
Load a pyrogram data of MP Calibration Standard



Auto-detection of RI

Creating a calibration file (1)

- **Select ”Add Rep. Files” and upload data files and input sample amount of each polymer.**
- **Then select Index level (different concentration) “2” and upload data files. You may use multiplier to automatically input sample amount of each polymer.**



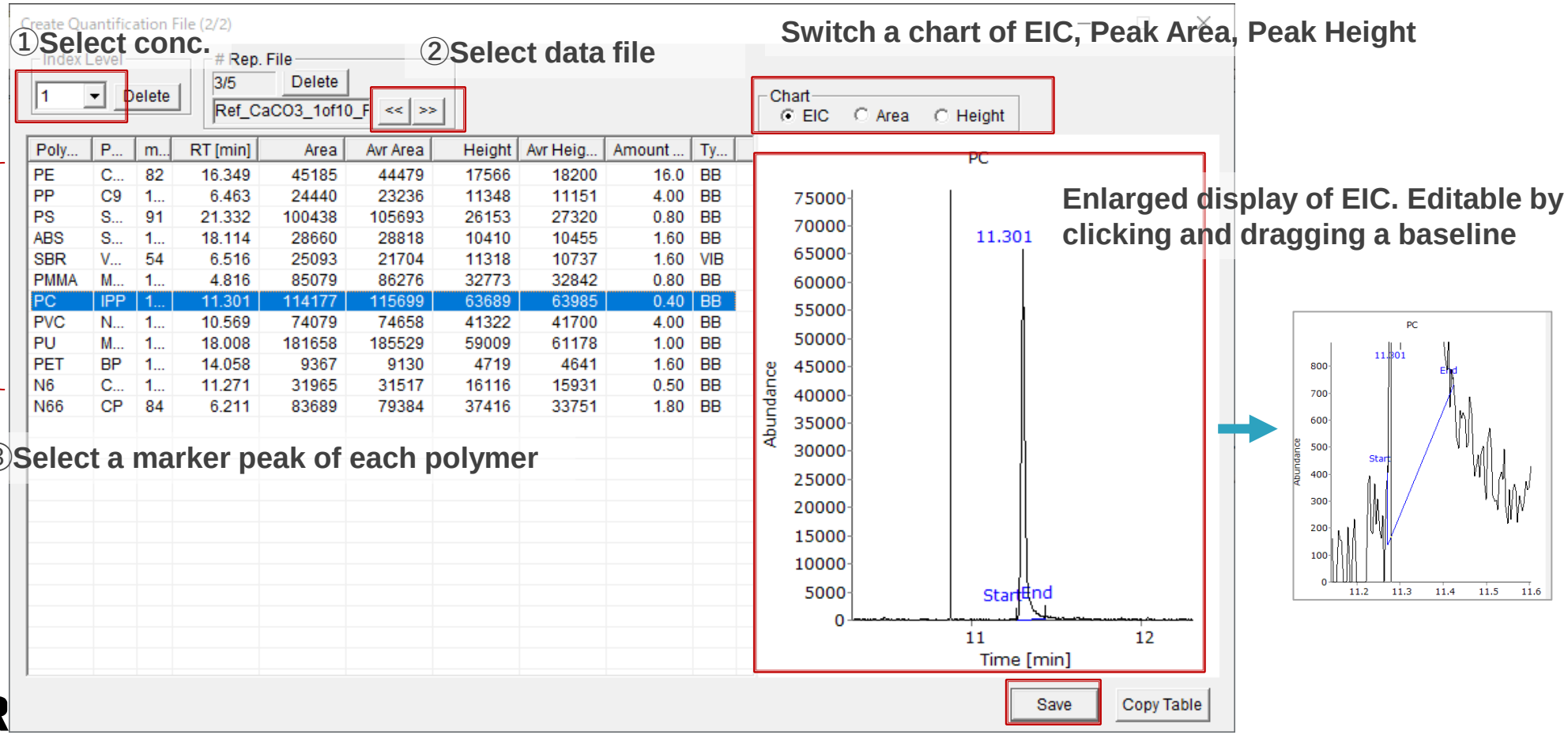
Load data file and input sample amount

Load data file of different conc.

Load data file of different conc.

Creating a calibration file (2)

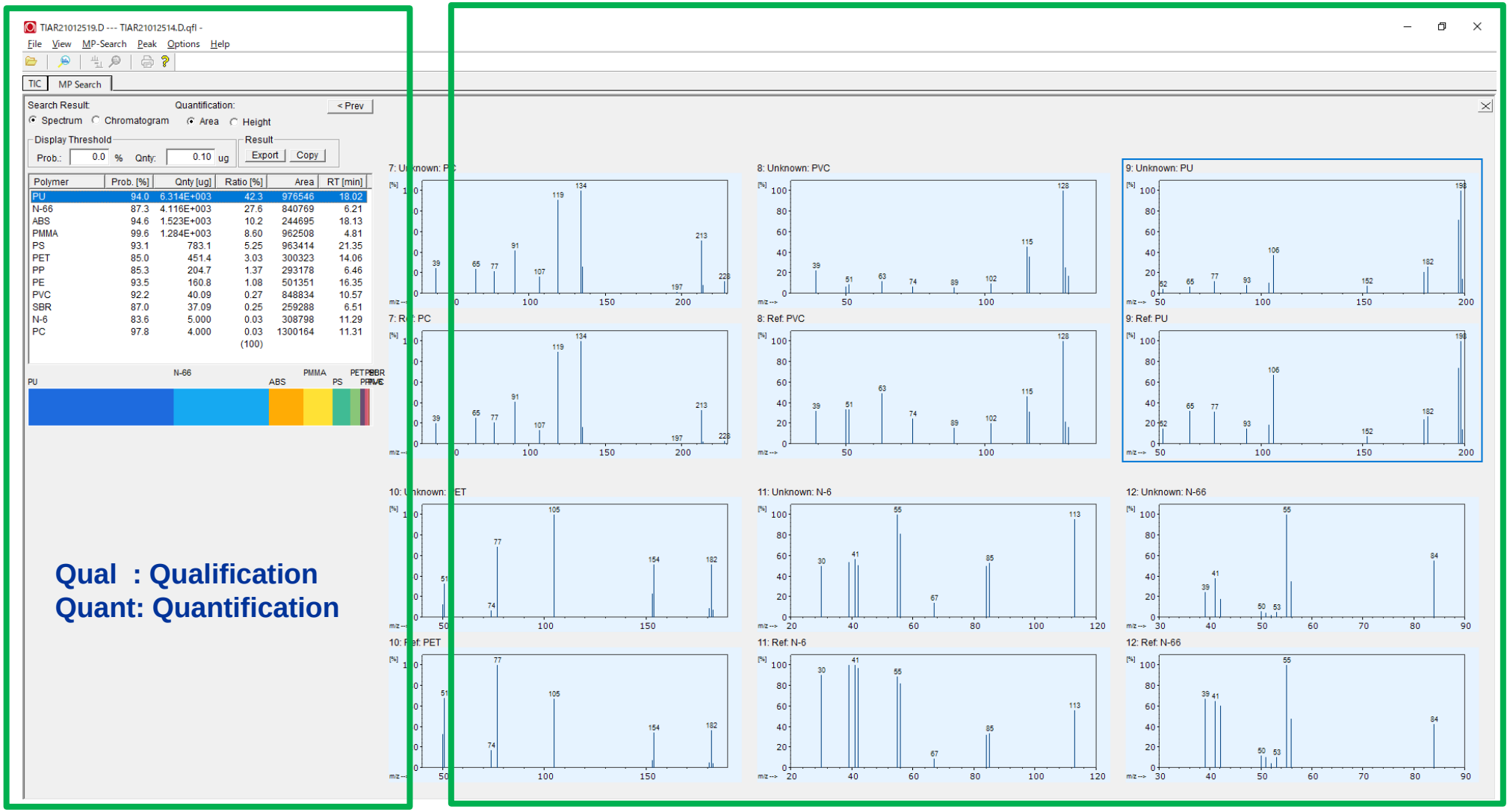
- Automatically creating a calibration file. If there is any problem with peak shape, peak area, peak shape and peak itself, correct it by clicking and dragging a baseline.
- Once correction is completed, select “Save”. A calibration file is created（qfl. file）.



Searching result (1/3) display overview

Results of Qual/Quant*

Mass spectrum and peak shapes



Qual : Qualification
Quant: Quantification

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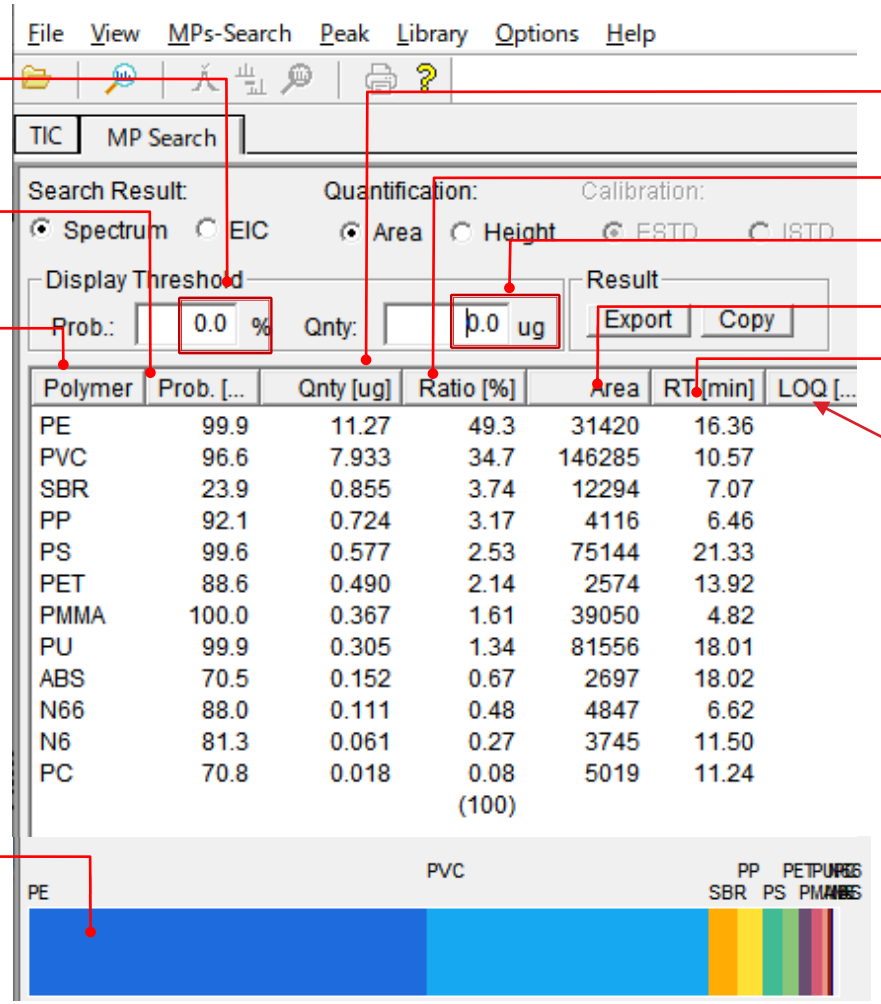
Searching result (2/3) display overview

Threshold of Match quality

Library Match Quality (%)

Plastic Name

Bar graph of Relative Amount (%)



Quant Result (µg)

Relative Amount (%)

Threshold of Quant result

Peak area

Retention time

LOQ (calibration, user)

Qual: Qualification
Quant: Quantification

It is noted that names of plastics in searching results suggest a polymer group.

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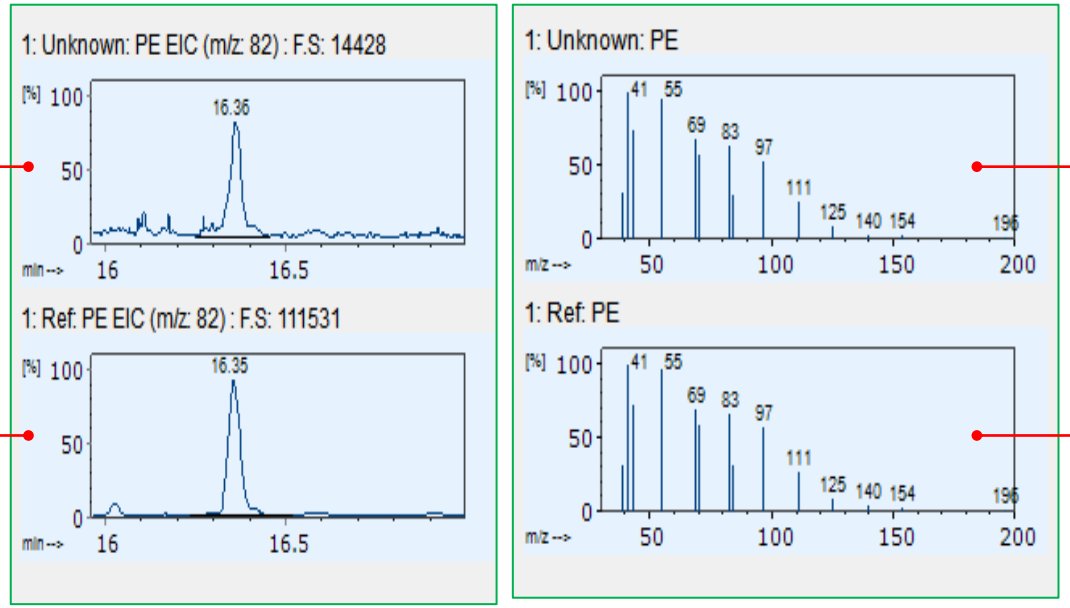
Searching result (3/3) Mass spectrum/Peak shape

Marker peak of Sample

Marker peak of MPs Calibration Standard for calibration



Mass Spectrum of Sample peak

Mass Spectrum of MPs Calibration Standard for calibration

- Each polymer has its own marker peaks and ions

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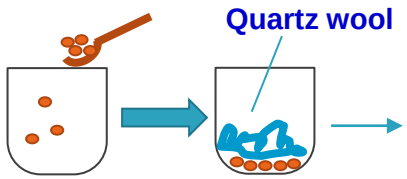
Sample prep for calibration procedure

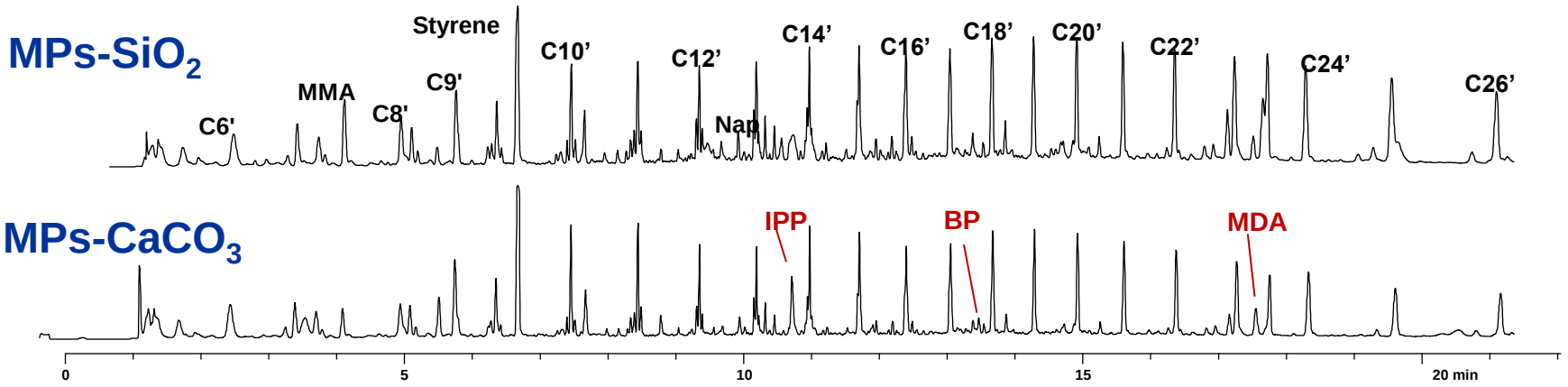
Put 4 mg of MP Calibration Standard into a sample cup



Py-GC/MS measurements

HR202i (A&D)
< Min: 0.01 mg >

The amount of each polymer in the 4 mg of reference mixture (PE: 160 µg, PP: 40 µg, PS: 8 µg, ABS: 16 µg, SBR: 16 µg, PMMA: 8 µg, PC: 4 µg, PVC: 40 µg, PU: 4 µg, PET: 16 µg, N6: 5 µg, N66: 18 µg, diluent: 3.8 mg)



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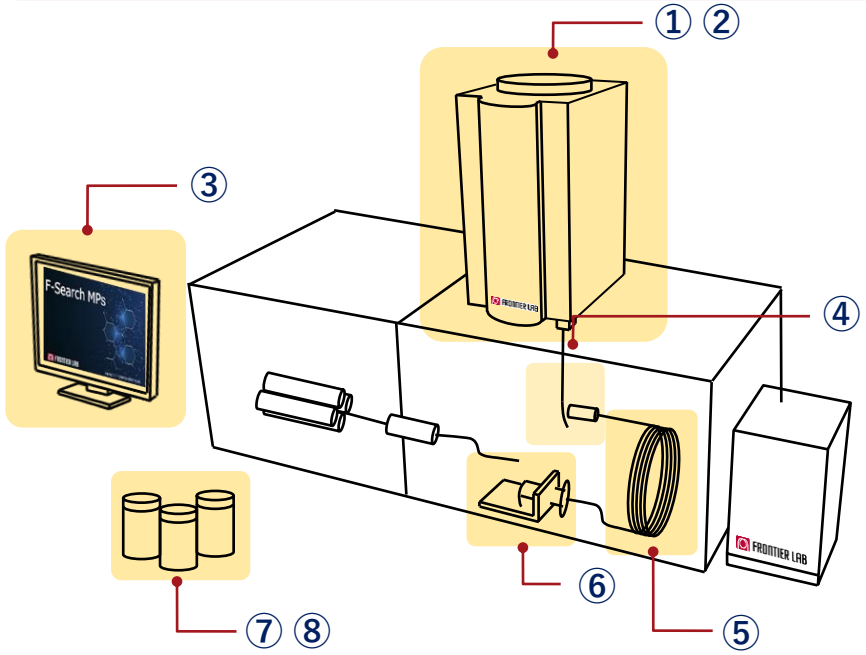
Summary & Questions ?



Micro Plastic Solution !

① Multi-Shot Pyrolyzer
② Auto-Shot Sampler
New ③ F-Search MPs 2.0
New ④ Packed GC Glass Insert
New ⑤ UAMP column kit
⑥ Vent-free GC/MS adapter
New ⑦ Microplastics Calibration Standard
New ⑧ Consumable parts set for MPs

UA: Ultra ALLOY®

- Combination with Single Q GC/MS system.
- Interpretation results by F-Search MPs 2.0 are optimized by Py-GC/MS system above.

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