

Why FPA IR Imaging is King in Microplastic Analysis

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The ubiquitous presence of microplastics is well documented. Numerous projects are currently assessing the extent of microplastic pollution and its potential risk to both humans and the environment. In these studies, the quantity, composition and size distribution of particles encountered, are the key factors. Efforts are also made towards a standardization of the principal analytical methods, but a general consensus has not yet been reached.^[1,2,3]

1. Methods for Microplastic Analysis

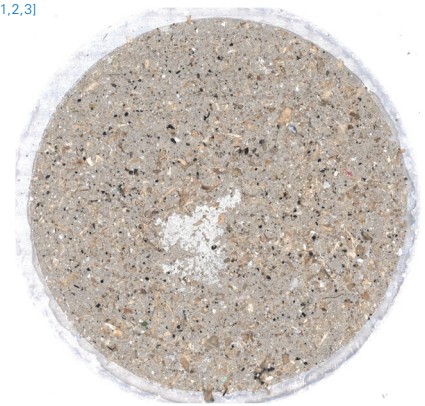
Basically, there are two main approaches to microplastic analysis:

- **Mass-focused** techniques are based on mass-spectrometry and provide exact mass distributions for the most common polymer types.
- **Particle driven** techniques focus on the number, identity and size distribution of particles and are based on different microscopic methods.

This whitepaper will focus on the latter.

The simplest method to locate particles is **visual microscopy**. While it can detect the presence of particles, it does not yield the particle composition, since it cannot distinguish between naturally occurring and synthetic particles. Nile red staining may help distinguishing plastic from non-plastic. However, the polymer type remains inaccessible and not every polymer can be stained.

- ➔ It must be emphasized, that the identification is the most important part in understanding the origin of the contamination. While visual microscopy might give a first indication, we need a method that not only finds particles, but also identifies them with high reliability.^[4, 5]



Real-life microplastic sample: Filter loaded with all kinds of sample material.

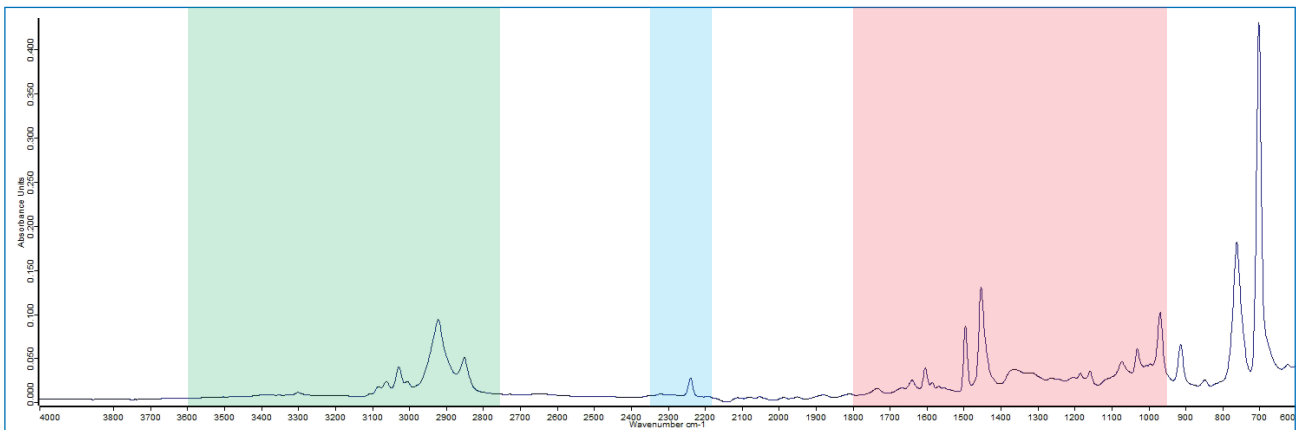


Figure 1: FT-IR spectrum of ABS. The spectral range accessible by QCL is marked in red. The nitrile band (2250 cm^{-1} , blue) is not accessible as well as the C-H and N-H region (green) that holds further important information about the particle identity.

Micro spectroscopy provides a basic solution to these problems. Here, microscopy is coupled with single-point spectroscopic methods such as Raman, FT-IR or IR laser spectroscopy. By individually analyzing particles, information on the chemistry and composition of the sample is obtained, allowing identification of the polymer.

- ➔ Unfortunately, single-point micro spectroscopy inherits the major drawback of visual microscopy and particles must first be visually recognizable before they can be analyzed at all. Therefore, tiny and transparent particles are easily overlooked and particle agglomerates, which are not properly separated, are usually detected as one big particle instead.

With the broad availability of **FT-IR FPA imaging technology**, the detection and identification of particles can be based solely on spectral information.

Assessing the complete sample like this, eliminates the need for prior particle detection, capturing every particle on the filter. Samples are measured at very high speeds, in their entirety and with a supreme spatial resolution.

- ➔ In this white paper we argue that the combination of micro FT-IR imaging and automated data analysis is the ideal solution to perform comprehensive, quantitative microplastic particle analysis. A fully automated measurement and evaluation approach represents the most effective and reproducible method there is to date, eliminating human error.^[6]

2. Plastic Identification by (FT)-IR

FT-IR spectroscopy is well-established when it comes to the identification of unknown substances. It is especially helpful for organic compounds, such as the polymers, microplastics are made of. Each polymer has its distinct absorption pattern of IR radiation and is easily identified by comparing this pattern to a reference.

Classical FT-IR is universally applicable and covers the mid-infrared region from $600 - 4000\text{ cm}^{-1}$, allowing the identification of virtually any existing polymer. Distinct features such as a nitrile band (around 2250 cm^{-1} , marked in blue) or C-H and N-H stretching bands ($2700 - 3600\text{ cm}^{-1}$, marked in green) are routinely available, aiding in the distinction of copolymers with the same backbone (e.g. PS/ABS/SAN).

IR techniques using a laser source (QCL) have a limited spectral range of about $950 - 1800\text{ cm}^{-1}$ (marked in red). They are therefore unable to detect the important spectral signatures in the higher wavenumber range.

3. FT-IR Measurement Techniques

In principle, there are three basic measurement techniques in FT-IR microscopy:

Transmission mode is the method of choice for IR transparent materials. This non-contact approach yields good spectral quality and fast measurement speed.

ATR delivers excellent spectral quality but requires contact. Because of this ATR-crystal, which serves as measurement interface, needs to be manually cleaned to prevent particle cross contamination. This is not only laborious but also makes automation difficult. In addition, harder particles such as glass, carbonates or sand can damage the crystal.

Transflection poses an even greater challenge. Since the IR light passes the sample twice, total absorption is reached twice as fast, limiting the maximum size of particles that can be identified. Furthermore, IR spectra in transflection are known to exhibit complex mixtures of specularly and diffusely reflected light that interfere with the identification of transflection spectra by ATR libraries.

4. Sample Preparation and Particle Recognition

After organic and inorganic residues (e.g. sand and plant material) have been removed by the initial pre-treatment, the particles are typically suspended in water and filtered onto a suitable IR transparent filter (e.g. aluminum oxide). Afterwards, contact-free measurements in transmission can be performed, delivering clean spectra ideal for evaluation.

- It must be mentioned, that during pre-treatment it is virtually impossible to remove all debris and residues from the environmental matrix. So still, not everything that is found on a filter, is also a microplastic particle.

For FPA IR imaging methods, this poses no problems, but for contrast-based methods (e.g. single-point Raman/IR) **this is a major drawback**, since the more residue remains, particles are harder to detect and might be missed entirely like the transparent particle in Figure 4. In the worst case, users waste time measuring non-plastic particles that end up falsifying particle statistics.

5. Assessing Applicability and Constraints

In general, visual (Fig. 2) particle recognition is highly unspecific and cannot distinguish between particles and surface alterations (e.g. fibers/scratches). Similarly, QCL methods based on the contrast of IR scattering at a single wavenumber also face difficulties. The dependence on an arbitrary threshold means that both cannot detect transparent or low-contrast particles.

- Commercially available single wavenumber scanning QCL systems show a strong drop in particle detection efficiency below 60 microns.^[10] These systems unpredictably **underestimate** the fraction of small particles.

Microplastic particles also tend to agglomerate in concentrated suspensions and often end up adjacent to each other on a filter. Here, unspecific contrast methods will recognize such agglomerates as a single particle, placing only a single measurement point, although several would be required (Fig. 3).

At low particle load, results are still decent, but with crowded filters (Fig. 4) **contrast-based methods fail entirely**. Accordingly, these techniques pose very high requirements towards sample preparation and may require complete removal of the sample matrix (e.g. sediment). Thus, dilution, aliquot sampling and preparation on dedicated sample carriers might be necessary.

- **Application of visual and QCL contrast methods is strongly limited.**

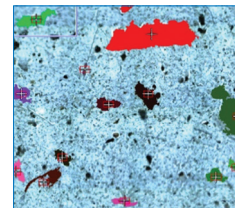


Figure 2: Individual particle recognition.



Figure 3: Particle agglomerate.

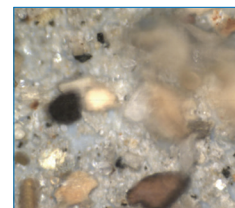


Figure 4: Densely loaded filter.



Figure 5: Dark microplastic on filter.

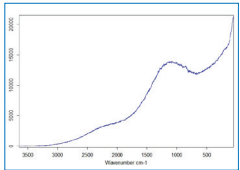


Figure 6: Fluorescence dominates the Raman spectra of the particle in Fig 5.

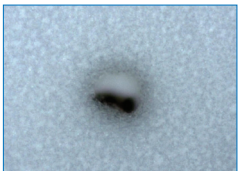


Figure 7: Filter and particle were burned during Raman measurement

6. The Role of Raman Spectroscopy

Due to the lack of array detectors, Raman micro spectroscopy relies on particle recognition by visual contrast and subsequent single-point Raman measurements. All above mentioned limitations of visual contrast-based particle detection apply, greatly increasing the time required to measure complete filters.

Still, Raman analyzes particles as small as $1\ \mu\text{m}$ while the diffraction limit restricts IR measurements to particles approx. $5\ \mu\text{m}$ in size.

For such small microplastics, however, the Raman signal to noise ratio is intrinsically low and the measurement parameters (excitation lasers, integration times, laser power, etc.) must be individually matched to the particle of interest, making automation impossible.^[4]

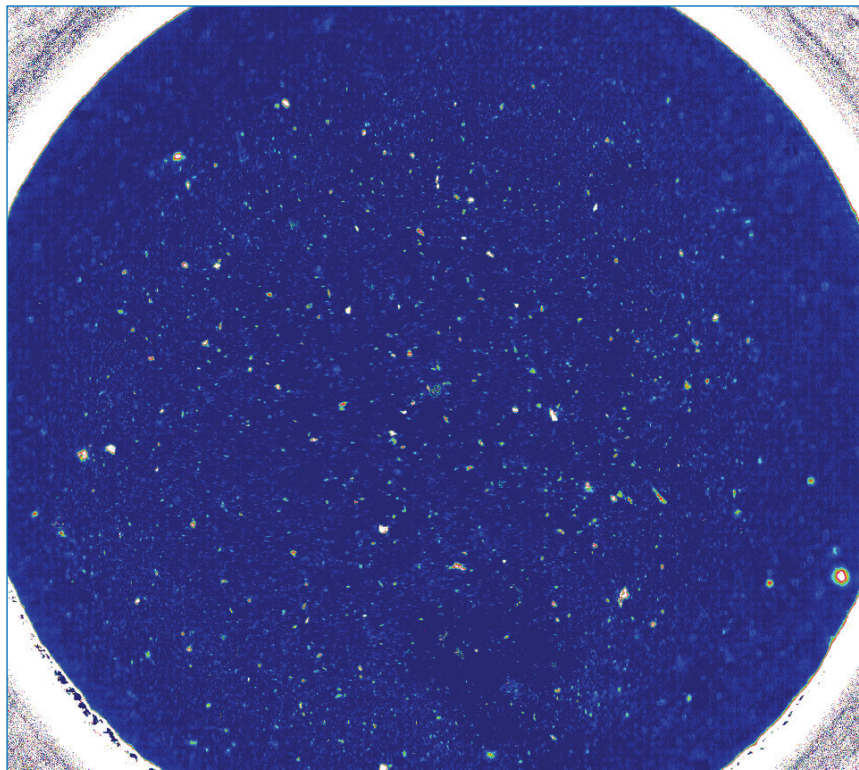
Further to that, Raman spectroscopy poses extremely high requirements towards sample cleanliness. Real environmental samples like the filter shown in Figure 8 (IR) and 13 (visual) cannot be measured with Raman.

This is for one due to the reasons concerning particle detection detailed in the previous section. Additionally, the measurement process is strongly affected by the presence of (organic) matrices which can lead to fluorescence or artifact signals. Eventually, Raman microscopy for microplastic analysis is restricted to a few, selected cases and broad applicability is prevented.

Reasons why Raman usually fails in particle analysis are:

- 1) Agglomeration and overlapping of particles
- 2) Raman spectra are affected by fluorescence effects (Fig. 6)
- 3) Dark particles being burnt by the laser, destroying the sample (Fig. 7)
- 4) Polymer fillers may obscure the base polymer spectrum
- 5) Difficulty distinguishing polymers with a similar backbone (e.g. ABS/SAN)

Figure 8: FT-IR image of a complete filter (diameter of $\sim 18\ \text{mm}$), measured with $5\ \mu\text{m}$ pixel resolution and $8\ \text{cm}^{-1}$ spectral resolution in ~ 3.5 hours.



7. Why FPA IR Imaging Shows No Drawbacks

FT-IR (FPA) imaging overcomes all these obstacles by omitting the visual detection of single particles altogether. True FPA IR imaging allows to measure entire filters (Fig. 8), in very short time. No visual selection required.

The evaluation is solely based on spectral data and ensures that all particles present on the filter are measured and evaluated. This method is not affected by particle load and tolerates presence of (organic) sample matrices.

The complete volume of **every particle** on the filter is analyzed. If particles overlap, they can be directly distinguished by their spectra, or, if they are made of identical polymers, they can be separated within the software. Figure 9 shows how FPA imaging automatically and correctly decomposes a complex agglomerate into individual particles. All other methods would fail to perform this separation.

Although many studies do not account for particles <300 μm , these are by far the most abundant. And the highest number is usually found in the range of 100 μm and below. [8,9] Capturing the full scope of the microplastics contamination therefore requires an analytical technique that can reliably detect and identify particles down to single-digit micrometer size.

FT-IR imaging with a **focal plane-array** detector (FPA) allows the collection of chemical images with high spatial resolution close to the diffraction limit (5 $\mu\text{m}/\text{pixel}$). It does this at an incredible speed of up to 900 spectra / second, making you find the smallest of particles in the shortest amount of time.

→ **Only an FPA can provide this much information at such great speeds.**

8. Limits of Detection and Automation

The detection limit describes the smallest particle that can still be detected by an instrument. To understand this, we must know about the diffraction limit and the role it plays in micro FT-IR imaging.

The diffraction limit is a wavelength dependent, physical phenomenon that describes the smallest structure that can still be fully resolved by an instrument or specific technique. Under ideal conditions, this is exactly half the wavelength. For FT-IR it is usually stated as 10 μm . However, to fully resolve these 10 μm , a pixel size of 5 μm or smaller is required according to the Nyquist criteria. That means that a focal-plane imaging detector is working at the edge of what is physically possible in microplastic particle analysis. But there is more to consider when evaluating the best possible analytical approach, namely automation.

→ Though the diffraction limit of FT-IR is about 10 μm , FPA imaging routinely detects particles smaller than 5 μm (Fig. 11) and delivers an excellent IR spectra quality that is ideal for identification purposes (Fig. 12).

Automation is key to bring microplastics analysis into standardization and while Raman microscopy might allow measurement of very small (sub-micrometer) particles, the analysis can never be automated.

Automation is also where single-point QCL measurements fall short. For small particles (< 60 μm) detection is not reliable, creating an unpredictable, systemic bias in the statistics. As this, however, is the most interesting size fraction in particle analysis, this method is unsuitable for automation.[10]

→ **This makes FT-IR imaging the best choice.**

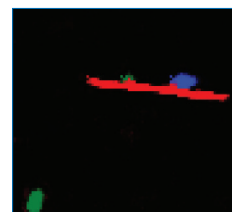


Figure 9: RGB image based on the FPA IR data acquired by LUMOS II. Particles are clearly separated.

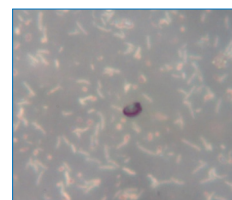


Figure 10: Visual image of a particle of 4x5 μm in size.

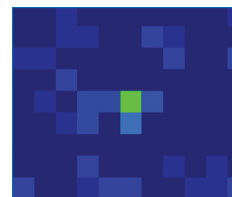


Figure 11: FPA detector image of the same particle (green, mid).

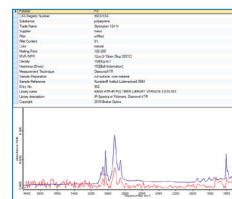


Figure 12: IR spectrum (red, bottom) of that individual pixel identified by a spectral library search.

9. Automated Measurement and Evaluation

Understanding the full scope of the global microplastics problem requires automated, reproducible procedures for the measurement as well as the evaluation. At the same time hardware should remain easy to use, give routine users more control, and must ensure intercompatibility.

By eliminating the pre-selection of particles, FPA imaging allows users of all skill levels to perform comparable measurements - with very little training. As far as evaluation goes, the most common approach is to compare the obtained spectral data with a reference library. Various libraries and matching algorithms are available for this purpose.

Different parameters can be selected to obtain a meaningful result and automation is possible.^[4] But for standardization it is crucial that the same measurement conditions and parameters are used every time to ensure comparability between different laboratories.

This can either be achieved by simply agreeing on a common reference data base, defined search parameters or by using a standardized machine learning model that was trained on a comprehensive set of reference spectra.

To summarize, automation is the key to standardization. With it, users of any skill level can operate instruments, collecting and analyzing FPA IR data in very little time, thus eliminating personal bias as a source for errors.^[6, 7]

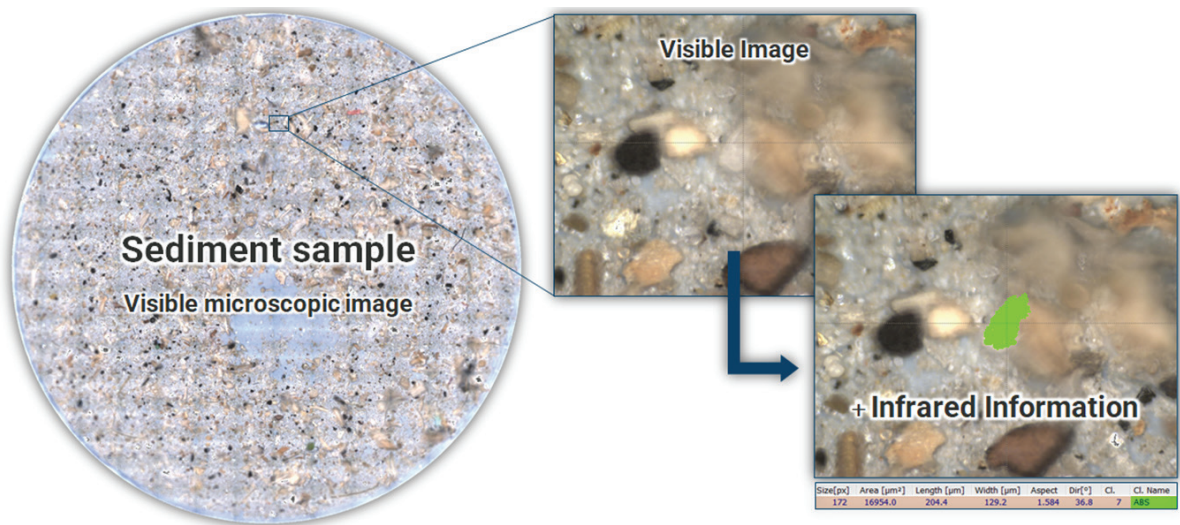


Figure 13: Example of a real environmental sample that was measured with FPA imaging. An ABS particle was identified based on the IR chemical image. For emphasis, the chemical image of the particle was superimposed on the visual image (green).

10. Real World Scenario: Sediment Sample

In an ideal scenario, measurements will give an excellent spectrum that naturally fits the reference. However, real world samples rarely match these sunshine scenarios and pose two major challenges:

- 1) Samples also contain other naturally occurring substances such as plant material, sand and remains of living organisms that have IR signatures.
- 2) The most abundant particles are also the smallest ones, making it difficult to reliably detect and identify them.

Contamination as stated in item 1) can be reduced by more elaborate sample pre-treatment, but it is not possible to remove it completely, especially when analyzing environmental or food samples.

PID /	Cent.X [µm]	Cent.Y [µm]	Size[px]	Area [µm ²]	Length [µm]	Width [µm]	Aspect	Dir[°]	Cl.	Cl. Name	Relevance	Similarity
02298	41543	26706	6	591.4	29.70	19.91	1.500	0.0	1	PP	0.4244	0.8943
09547	40346	31495	13	1281.0	63.09	31.93	1.986	17.5	3	PVC	0.6082	0.5562
09621	44206	24625	45	4436.0	98.98	78.15	1.259	87.2	3	PVC	0.7810	0.7621
11734	40266	29235	37	3647.0	98.52	68.22	1.449	154.9	4	PU	0.7964	0.5744
11736	40336	29165	7	690.0	37.16	28.51	1.300	120.5	4	PU	0.7581	0.5098
13678	43474	27453	109	10744.0	208.9	79.38	2.646	175.0	5	PET	0.7423	0.7670
13779	44969	22016	132	13011.0	177.6	109.7	1.618	136.6	5	PET	0.6996	0.7931
13826	45454	23470	244	24051.0	237.2	171.6	1.383	42.6	5	PET	0.7931	0.7979
15430	38554	19826	172	16954.0	204.4	129.2	1.584	36.8	7	ABS	0.7344	0.7207
16687	34515	20433	307	30261.0	287.5	170.4	1.694	155.2	10	PMMA	0.6207	0.8191
16817	45533	23589	88	8674.0	149.9	101.0	1.493	2.1	10	PMMA	0.4573	0.6936
17412	37356	26845	50	4929.0	124.5	78.09	1.603	11.5	12	CA	0.5309	0.5528
19401	33594	30171	6	591.4	39.60	19.91	2.000	0.0	13	EVAc	0.3511	0.8429
19402	33614	30111	15	1479.0	52.20	42.67	1.230	170.6	13	EVAc	0.3724	0.8288
19416	33703	30291	10	985.7	54.41	36.70	1.478	117.8	13	EVAc	0.3200	0.7705
19419	33772	30271	14	1380.0	79.17	30.37	2.622	178.6	13	EVAc	0.2914	0.7557
24116	41365	24247	32	3154.0	72.26	68.13	1.066	13.1	21	PLA	0.5233	0.5073

Again, single-point Raman or FT-IR measurement approaches based on the visual detection are not feasible for real, environmental samples.

Figure 13 shows a Ø25 mm Anodisc filter with a filter area of ~300 mm². It was measured in its entirety with FPA FT-IR imaging at 5 µm pixel size and 8 cm⁻¹ spectral resolution, taking ~3.5 hours @ 750 IR spectra per second. Techniques other than FPA imaging, which cover the entire spectral range, would require several days (~60 h) while providing inferior spatial resolution.

The IR data was analyzed with the Microplastics Finder (by Purency), yielding a detailed particle list and a statistical report (Figures 14 and 15). Although particles are partly sandwiched between plant material and a sand layer, the identification is successful and gives pixel accurate size information.

11. Conclusion: FPA FT-IR Imaging is King.

The critical analysis of single-point Raman, FT-IR, and QCL approaches clearly shows that these methods are serviceable if samples are clean, particle size is not too small and particle load is sufficiently low, requiring aliquotation in some cases. Automation is also possible to a certain degree but comes with severe limitations in regard to spectral quality (Raman) or particle size (QCL).

The detection limit given for single point FT-IR is usually 10 µm, and 60 µm for single point QCL - if measurements are automated. Measurement time increases with the number of particles and can reach 7-8 hours per sample.

In case of QCL transfection, identification is not reliable due to comparison with spectra of a different technique. These methods fail entirely when particles are adjacent or overlapping or when residue from the sample matrix (like a river sediment) is present.

While Raman can measure particles down to 1 µm, it is not possible to reliably automate the process. Many physical effects can prevent successful measurements or identification altogether and different particles even require different parameter settings.

Figure 14: List of all identified particles with their size information.

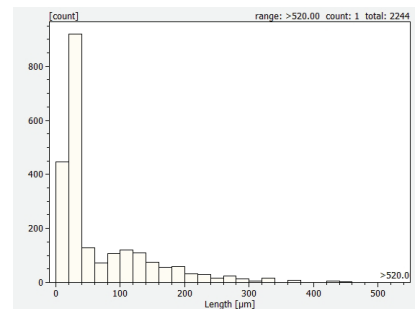


Figure 15: Statistical size distribution overview of found particle on the filter.



Figure 16: LUMOS II FT-IR microscope on the left and FPA imaging version on the right. It allows analysis in transmission, reflection and ATR.

Ultimately, FPA FT-IR imaging is king because:

- 1) Particle detection is independent from visual contrast
- 2) Identification is unaffected by polymer type, fillers, or sample matrix
- 3) Complete filters are covered at max-speed and best spatial resolution

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