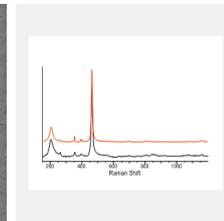
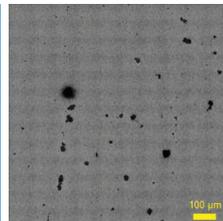


## Raman Spectroscopy

# Statistical, morphological and chemical characterization of particles on cellulose nitrate filters for quality control



Application Note  
Pharmaceutical RA58

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### Abstract

Statistical and morphological analysis of particles on a filter has been complemented by chemical analysis based on Raman spectroscopy using the ParticleFinder tool, allowing further insight into the particle composition and its origin within a production line.

### Key words

Particles, Filter, Contaminant, Statistics, Morphology.

## Introduction

Particles can be analyzed for their morphology (size and shape), and statistics (count, and size distribution). The traditional analysis methods such as static/dynamic imaging and laser diffraction do not include chemical analysis information, and at times that limits the usefulness of the analysis.

For example, a pharmaceutical preparation for injection should not contain more than a specified number or size of particles. Traditional analysis is sufficient to qualify the sample for pass or fail per specifications, however, it may not be enough to identify the source of excessive particles, and allow optimization of the process to eliminate them. Chemical characterization of the particles using Raman spectroscopy analysis can help trace the origin of failure. This can lead to improvements in manufacturing efficiency, and thus reduce the cost of manufacturing.

Another example is the monitoring of the cleaning process. When multiple products share a manufacturing facility, it must necessarily be cleaned between production runs. One way to ensure the cleaning is effective is to monitor swabs and filter equipment rinses after the wash, and examine the residual particles. As with the example above, the count of particles may indicate whether any further cleaning is required, and the shape and chemical identity of particles may indicate where or what kind of further cleaning is necessary.

In this application note, we present ParticleFinder for LabSpec 6 as a tool for easy and efficient statistical and combined chemical analysis of particles.

## ParticleFinder

The ParticleFinder module couples static image analysis of particles with automated Raman micro-spectroscopy, allowing large numbers of particles to be located, counted/sized and analyzed for chemical composition. Among morphological parameters that can be generated by ParticleFinder to characterize the size and shape of a particle are area, perimeter, the length of the major axis (the longest line that can

be drawn within the particle), and the length of the minor axis (the line drawn perpendicular to the major axis within the particle). Other parameters if needed can be derived from these parameters. The Raman spectra can be acquired with automated optimization routines (including autoexposure and autofocus), and subsequently analyzed with advanced Raman analysis packages such as LabSpec 6 for detailed spectroscopic and chemical information.

## Experiment

The sample was a circular cellulose nitrate filter (47 mm diameter) through which a fluid sample had been filtered. The requirement was to analyze particles retained on the filter. The desirable results were particle counts, size distribution, and material analysis.

Spectra were recorded with a HORIBA Raman microscope, using the LabSpec 6 ParticleFinder module for particle location, morphological analysis and automated spectrum acquisition. Subsequent data processing was performed using LabSpec 6 as well, with chemical identification made through the HORIBA Edition of KnowItAll® (Bio-Rad Laboratories, Informatics Division).

A montaged wide field of view video image was acquired, covering a 1 mm x 1 mm area. On the filter particles appeared darker than the filter in the image, allowing an upper threshold to be used to locate the particles. A size threshold was applied to exclude very small particles (diameter <5 µm). There were 58 particles in the image (Figures 1 and 2). Table 1 below summarizes the statistics of measurable parameters of particles observed in the image.

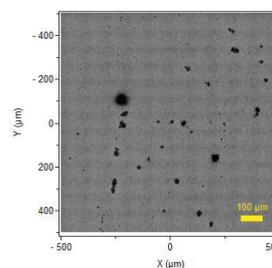


Figure 1. A video image of the sample, 1 mm x 1 mm

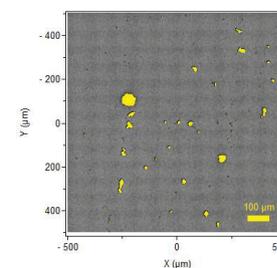


Figure 2. Localization of 58 particles (diameter ≥5 µm)

	Area ( $\mu\text{m}^2$ )	Perimeter ( $\mu\text{m}$ )	Major axis ( $\mu\text{m}$ )	Minor axis ( $\mu\text{m}$ )
Average	253.59	72.33	17.29	13.00
StDev	486.6	80.6	15.0	10.3

Table 1: Statistical summary of measurable particle size/shape descriptors

Five more images, each covering 1 mm x 1 mm, were acquired and processed similarly to the image above, yielding combined results from the six images comprising 256 particles in total. Even though these represent a relatively small portion of the entire filter, they are sufficient to demonstrate the routine analysis procedure for samples like this.

In the next step, a Raman spectrum was obtained from the center of each particle. All spectra were preprocessed as necessary (e.g. baseline correction, normalization), and then compared to reference spectra to identify the chemistry.

Note here that a traditional Raman imaging analysis approach would require collecting spectral data from every point on the optical image, the vast majority of which are irrelevant (being filter, not a particle of interest). By using the ParticleFinder module, spectra from only the particles were collected, greatly reducing the total duration of the measurement.

## Results

256 particles were located and analyzed across the six 1 mm x 1 mm areas. The chemical assignment of spectra is described below.

### Quartz

Out of 256 particles, 196 were identified as quartz ( $\text{SiO}_2$ ), with size/shape descriptors as shown in Table 2 below.

	Diameter ( $\mu\text{m}$ )	Ratio	Circularity
Median	7.85	0.84	0.67
StDev	6.39	0.17	0.11

Table 2: Statistical summary of size/shape descriptors for quartz particles

A particle spectrum (black) is shown below (Figure 3) in comparison to a quartz ( $\text{SiO}_2$ , red) reference spectrum.

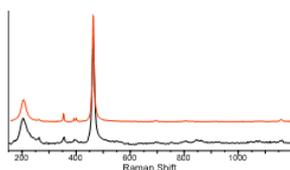


Figure 3: Comparison between a particle spectrum (black) and the reference spectrum of quartz crystal (red)

### Quartz and rutile

There were ten more quartz particles that show spectral features of rutile ( $\text{TiO}_2$ ) as well. A particle spectrum (black) shows a good agreement with the composite spectrum (red) of rutile and quartz reference spectra.

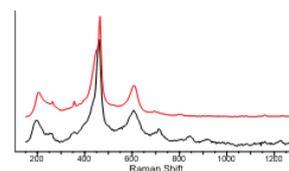


Figure 4: Comparison between a particle spectrum (black) and the reference spectrum being a sum of the spectra of quartz and rutile

These particles tend to be bigger than quartz particles (see Table 3), and may be conglomerates of two materials.

	Diameter ( $\mu\text{m}$ )	Ratio	Circularity
Median	12.49	0.85	0.73
StDev	3.98	0.10	0.10

Table 3: Statistical summary of size/shape descriptors for quartz/rutile particles

### Other minerals

In addition to the dominant quartz particles, there were single instances of rutile, dolomite ( $\text{CaMg}(\text{CO}_3)_2$ ), and albite particles.

	Diameter ( $\mu\text{m}$ )	Ratio	Circularity
Rutile	38.19	0.93	0.59
Dolomite	7.11	0.63	0.43
Albite	10.49	0.64	0.49

Table 4: Statistical summary of size/shape descriptors for rutile, dolomite and albite particles

The rutile particle is much bigger than quartz particles, and the spectrum (Figure 4), shows the mixed features of rutile and butyl rubber. This could be a conglomerate of rubber and rutile.

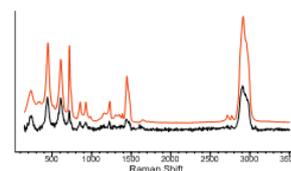


Figure 5: Comparison between a particle spectrum (black) and the combined spectrum (red) of reference spectra of rutile and polyiso-butylene

The dolomite particle is similar in size to quartz particles, but less circular (low ratio and circularity). The spectrum (black) shows a good agreement with the reference spectrum (red) of dolomite (Figure 5).

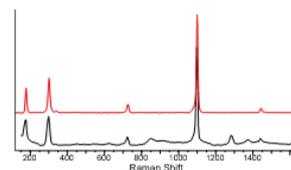


Figure 6: Comparison between a particle spectrum (black) and a reference spectra of dolomite (red)

The albite particle is a little bigger than quartz particles, and less circular. The spectrum (black) shows a good agreement with the reference spectrum (red) of albite.

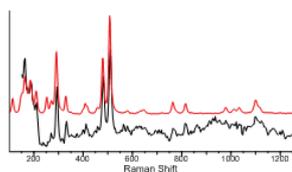


Figure 7. Comparison between a particle spectrum (black) and a reference spectrum of albite (red)

### Other

There were 39 particles that show the filter spectrum. Smaller than quartz particles, they could be transparent materials with weak or no Raman scattering intensity such as silica glass. Alternatively, since they were analyzed only at “center of mass” it is possible that the particle material was missed due to the particle shape.

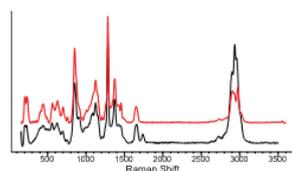


Figure 8. Comparison between the filter spectrum (black) and a reference spectrum of nitrocellulose (red)

39 Particles	Diameter (μm)	Ratio	Circularity
Median	6.80	0.81	0.55
StDev	2.51	0.15	0.17

Table 5. Statistical summary of size/shape descriptors for particles showing filter spectrum characteristics

There were eight particles that show high background or no Raman features, and could not be identified.

### Morphological characteristic statistics

The area, A, and the perimeter, P, are size descriptors, and measurable parameters. The area equivalent diameter,  $\chi_A = \sqrt{(4A/\pi)}$ , is a derived parameter, and one of the most commonly used size descriptors. Their distributions are shown below (Figures 9).

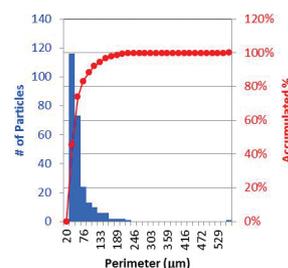
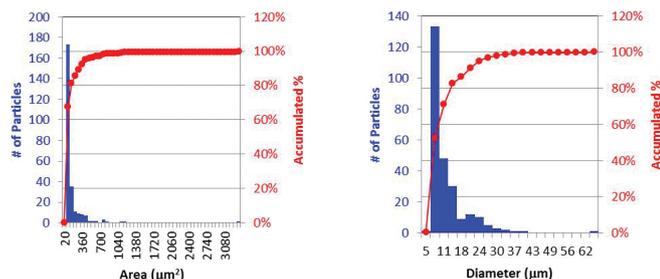


Figure 9. Statistical summary of area, area equivalent diameter and perimeter for 256 particles

The mean, median and standard deviation of diameters are used to characterize/represent the ‘batch’ or the collection of all particles. Other parameters that are commonly used are  $d_{10}$ ,  $d_{50}$ , and  $d_{90}$ .

Mean (μm)	10.57	Median (μm)	7.83	StDev (μm)	7.09
$d_{10}$ (μm)	5.47	$d_{50}$ (μm)	7.82	$d_{90}$ (μm)	19.9

Table 6. Mean, median and standard deviation values of diameters for all 256 particles, with additional  $d_{10}$ ,  $d_{50}$ , and  $d_{90}$  parameters.

The shape of a particle is irregular and arbitrary, and often approximated as a circle/sphere or an ellipse/ovoid. When a particle image is a circle, the perimeter derived from the area equivalent diameter,  $\chi_A$ , should be identical to the actual particle perimeter,  $P_m$ . The ratio  $P_m / (\pi\chi_A)$  is called Circularity, C, and 1 for a perfect circle. Lessened Circularity,  $C < 1$ , indicates the particle shape deviates from a circle.

Below are distributions of aspect ratio and Circularity. When a particle is approximated as an ellipse, one can measure the lengths along the major axis and the minor axes. The aspect ratio,  $R = (\text{Length of the major axis}) / (\text{Length of the minor axis})$ , can describe the shape of the particle. For example,  $R = 1$  for a circle,  $R < 1$  for an ellipse, and  $R \ll 1$  for a needle.

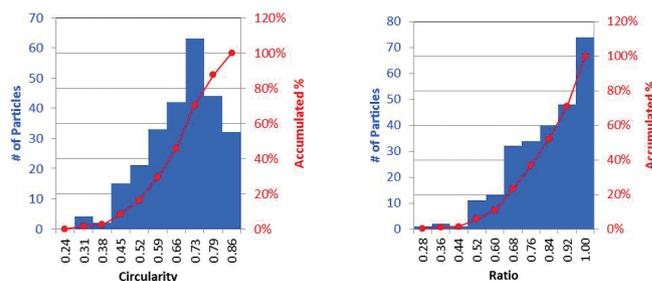


Figure 10. Statistical summary of circularity and ratio for all 256 particles

Statistical summary for the entire sample set (six images) is shown below.

	Ratio	Circularity
Mean	0.81	0.65
Median	0.83	0.67
StDev	0.16	0.13

Table 8. Mean, median and standard deviation values of ratio and circularity for all 256 particles

Median is more popular to represent the entire particles as a group than the average because of its relative insensitivity to outliers. For example, the mean of the area changes from 123.70  $\mu\text{m}^2$  to 114.66  $\mu\text{m}^2$  when the largest particle is excluded while the median changes from 48.18  $\mu\text{m}^2$  to 48.06  $\mu\text{m}^2$ .

## Conclusions

256 particles retained on a filter have been analyzed both morphologically and chemically using a HORIBA Raman microscope and LabSpec 6 Spectroscopy Suite. ParticleFinder, a dedicated particle analysis module in HORIBA's LabSpec 6 software, was used to record a large area (1 mm x 1 mm), identify particles, select particles per the user defined criterion (in this case, the area equivalent diameter  $\geq 5 \mu\text{m}$ ), analyze morphology of particles, and record Raman spectra of each particle, automatically. The results show the majority of particles are quartz with a few other materials including rutile, albite and calcite. The fact that the majority of the particles are mineral can point to the source of the contamination. Effort on reducing impurities should focus on quartz either as an impurity in the raw material or from damaged process equipment.

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