application note

# Foreign Particulate Matter testing using the Morphologi® G3

## Introduction

The Morphologi® G3 with its Foreign Particle Detection capabilities allows the detection, enumeration and size classification of foreign particulate matter collected on a filter, by automated image analysis.

Many industries have to perform development and quality control testing procedures that involve the enumeration of foreign particulate matter (FPM). In the Pharmaceutical industry such testing is particularly important for example, USP 788 details the specification of acceptable levels of FPM that can be found in injectable drugs. The FDA have produced guidance documentation that provides regulatory recommendations for foreign particle testing in Metered Dose Inhalers (MDIs), Dry Powder Inhalers (DPIs) and Nasal Spray and Inhalation Solution, Suspensions and Spray Drug Products. Testing for FPM in Orally Inhaled and Nasal drug products (OINDPs) is also discussed by the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS)[1, 2]

FPM are contaminant particles that can typically derive from the active or excipient material or from the administration device, for example from abrasion or shearing during activation. Types of FPM include glass, transparent synthetic fibers, stainless steel, rubber, aluminum and plastic particles.

Traditionally analysis by light microscope based techniques normally involve an operator manually detecting and sizing particles. This is not only time consuming but may also be open to subjectivity. Automation using the Morphologi G3 allows fast, reproducible analysis of FPM and provides a vast amount of information on every particle detected. This application note explains some of the features employed for FPM analysis and describes testing of an inhaled product as an example.

## Morphologi G3 features allowing FPM detection

### Filter holder

Special glassless sample carrier plates are available for mounting a prepared filter on to the Morphologi G3 instrument. Their special design ensures the filter paper is stretched flat, which is essential for automated analysis. Figure 1 shows the 47 mm filter holders.

**Figure 1:** The 47 mm filter holders

The filter holders allow two filters to be presented to the instrument and therefore analyzed successively without any manual intervention. Figure 2 shows the 25 mm filter holder mounted on the Morphologi G3 instrument.

Glassless carriers eliminate the risk of particles on the glass surface being counted, they prevents particles from being crushed by the glass and simplify cleaning requirements. However, glass slide carriers are also available for cases when a sample preparation procedure has been developed using this method.

**Figure 2**: Morphologi G3 instrument with the 25 mm filter holder in place on the stage**.**

### High Resolution

The Morphologi G3 software supports Episcopic illumination as well as Diascopic in the standard operating procedure (SOP) which allows automated analysis of particulate matter collected on filter papers.

The combination of high quality microscope objectives with high numerical apertures, along with a 5 Mega Pixel scientific grade camera allows confident analysis of particles down to 2 μm in size. Even for such small particles images contain a sufficient number of pixels in them when measuring with a 10 X magnification to be confident that they are particles. The pixel size at the camera is 2.8 μm thus, when combined with the 10 X magnification the effective pixel size is 0.28 μm so a particle that has a Circular Equivalent Diameter of 2 μm would contain approximately 35 pixels.



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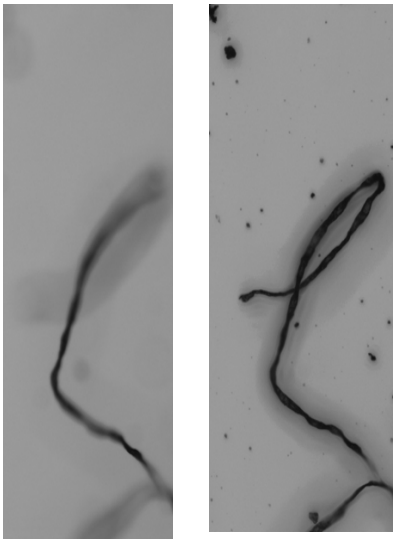


**Figure 3:** Sections of a field of view frame without (left) and with (right) z-stacking.

## Z- Stacking

If required the system can perform z-stacking so images of each frame are taken at different focus positions and combined together before the particles are separated from the background. This allows large particles to remain in focus and also ensures that any small particles that may be in the well of a filter paper (after filtration though a mesh) are counted. Figure 3 shows a section of a frame containing a fibrous particle with and without z-stacking.

## Particle stitching

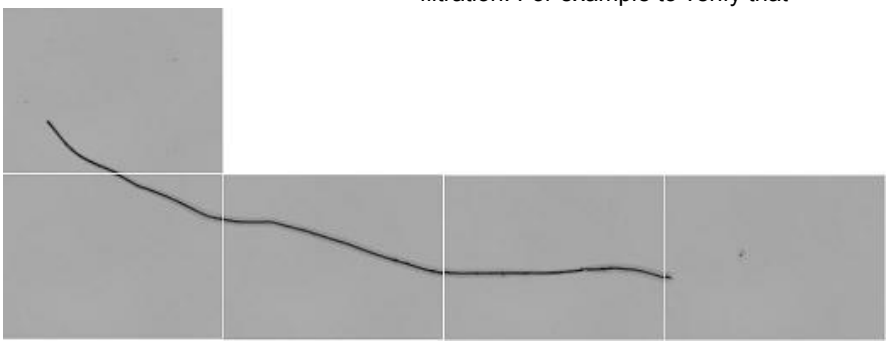
If particles cross two or more frames, as is typically the case for fibers, the software identifies the frames which contain the particle image and effectively stitches the pieces of the particle together and then extracts the whole edge stitched particle from the background. It then calculates all of the morphological parameters for that particle image as shown in figure 4. The particle shown is longer than 4 mm and has been extracted from several frames and then edge stitched together. Without particle edge stitching the longest particle to be certain of detecting with the 10 X magnification was 420µm.

## Dual Threshold

Both highly reflective particles such as metal and dark particles such as rubber can be detected by implementation of a dual threshold. This also means particles that contain both light and dark regions are also detected as one single particle rather than being fragmented into different parts as shown in figure 5.

## Composite Image

At the end on each analysis a composite image of the whole of the scanned area is automatically generated. This is useful to verify that there were no faults in the filter paper itself, such as a crease that may have affected the analysis. Both the composite image and the X-position verses Y-position scatter plot provided by the Morphologi software allow visualization of the position of particles of particular interest on the filter (figure 6). Additionally, they can be used to assess the quality of the filtration. For example to verify that



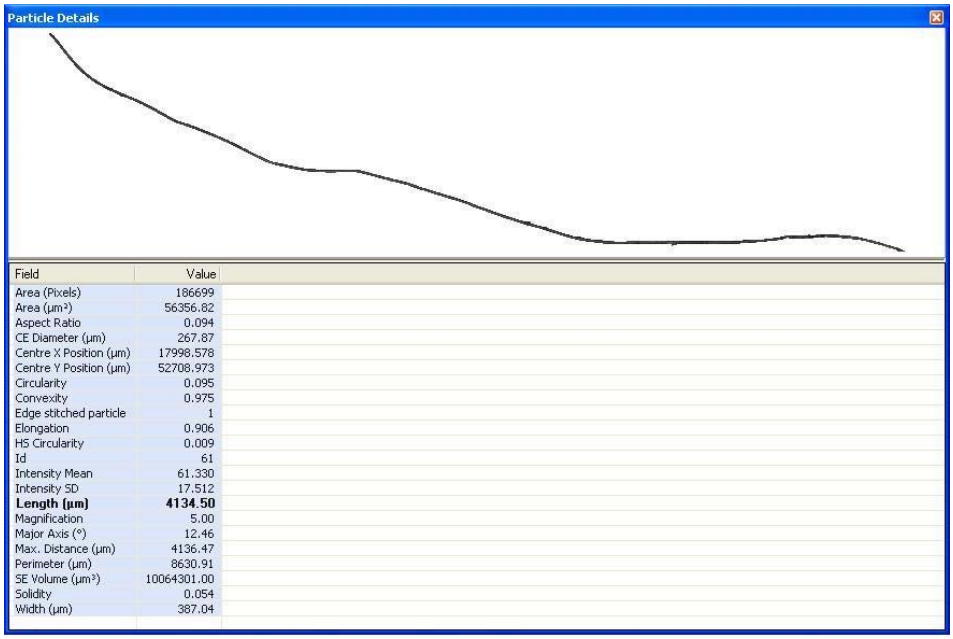



**Figure 4:** Field of view images of a particle overlapping several frames and the resulting edge stitched image with particle details. Note length of fiber is 4.1 mm.

the particles are evenly distributed over the filter and not limited to the edge of the wetted area.

## Example: Enumeration of FPM in inhaled products.

FMP testing during the development of an inhaled product and for quality control requires that particles falling into the following size classes are counted:

Particles with length ≥ 2 < 10 μm

Particles with length ≥ 10 < 25 μm

Particles with length ≥ 25 < 100 μm

Particles with length >100 μm

Specifications for the number of particles allowed in each class for inhalation drug formulations are usually established during development studies and are often based on rigorous statistical analysis of process batch history data, stability batch data and safety considerations.[2]

In order to analyze the filter samples on the Morphologi G3, a standard operating procedure (SOP) was developed to count particles and to define them into the specified size classes using the classification feature. It is easy to set up alternative size classes either pre or post analysis if required.

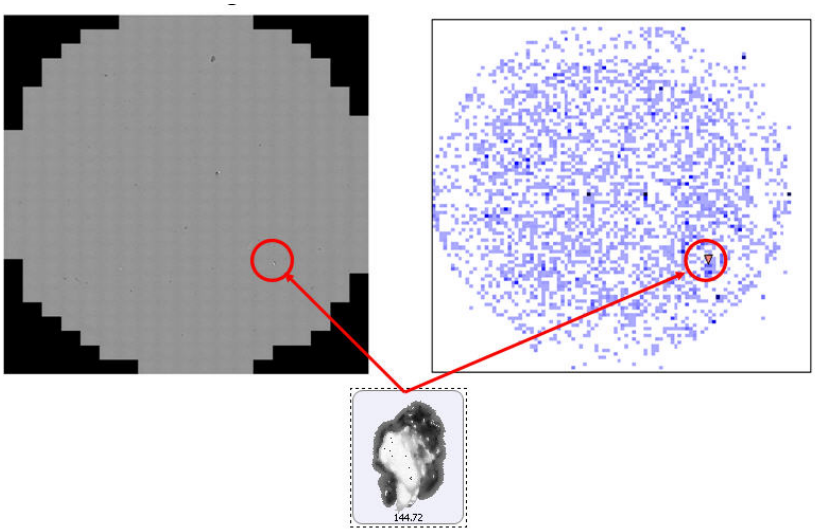



**Figure 6:** Composite image and X position Vs Y position scattergram which show the position of particle of interest on the filter.

The inhaled product was sampled and suitable solvents were used to selectively dissolve the active pharmaceutical ingredient and excipients. The resulting solution was then filtered through a mixed cellulose ester filter that captured the remaining FPM. All preparations were performed in a particle-free atmosphere.

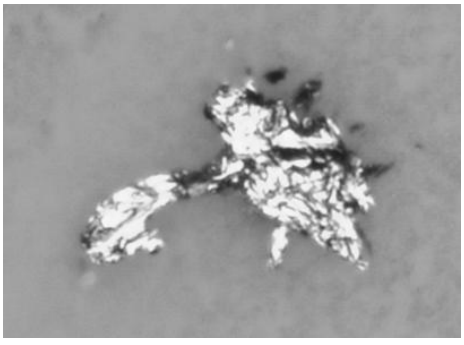
Once dry, the filter was placed in the Morphologi filter holder and onto the automated stage. The wetted area of the filter paper in this case was 10 mm in diameter. A circular scan covering the whole wetted area with z-stacking took approximately 20 minutes.

At the beginning and end of every measurement a verification of the light intensity is performed and recorded to ensure reproducible results. Also a pixel calibration is always carried out on the NPL (NIST) certified gratings that are integrated in the XY stage.

Instrument repeatability is demonstrated in Table 1 where six repeat measurements were carried out on the same filter sample. The same sample was also measured 3 times each day for 3 consecutive days. The results are shown in table 2.

To ensure orientational independence of the filter paper a filter sample was analyzed four times and in between each measurement the filter was rotated by 90 degrees. Table 3 present the results of this analysis.

In each case high reproducibility and repeatability is demonstrated.



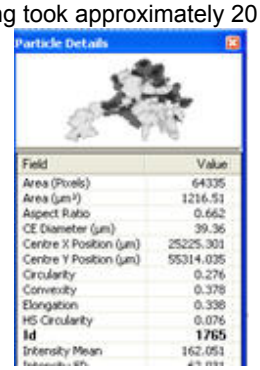



**Figure 5:** Field of view image of a particle containing both bright and dark region and the Morphologi image of the particle along with particle details.



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**Table 1**: Results of repeatability test of where six repeat measurements were performed on the same filter.*

| Sample Name | Total Particles | 2-10µm | 10-25µm | 25-100µm | >100µm |
|---|---|---|---|---|---|
| Filter B Scan 1 | 13893 | 13025 | 712 | 146 | 10 |
| Filter B Scan 2 | 13853 | 12976 | 714 | 153 | 10 |
| Filter B Scan 3 | 13945 | 13066 | 720 | 152 | 7 |
| Filter B Scan 4 | 14211 | 13334 | 716 | 152 | 9 |
| Filter B Scan 5 | 13952 | 13108 | 686 | 149 | 9 |
| Filter B Scan 6 | 13855 | 12982 | 711 | 152 | 10 |
| **Mean** | 13952 | 13082 | 710 | 151 | 9 |
| **Standard Deviation** | 134 | 133 | 12 | 3 | 1 |
| **RSD %** | 0.96 | 1.02 | 1.71 | 1.76 | 12.75 |

**Table 2**: Results of repeatability test of where a sample was measured 3 times each day for 3 consecutive days.*

| Sample Name | Total Particles | 2-10µm | 10-25µm | 25-100µm | >100µm |
|---|---|---|---|---|---|
| Filter B Scan 1 Day 1 | 13893 | 13025 | 712 | 146 | 10 |
| Filter B Scan 2 Day 1 | 13853 | 12976 | 714 | 153 | 10 |
| Filter B Scan 3 Day 1 | 13945 | 13066 | 720 | 152 | 7 |
| Filter B Scan 1 Day 2 | 13820 | 12935 | 713 | 162 | 10 |
| Filter B Scan 2 Day 2 | 14262 | 13358 | 736 | 158 | 10 |
| Filter B Scan 3 Day 2 | 13896 | 12984 | 745 | 156 | 11 |
| Filter B Scan 1 Day 3 | 14216 | 13315 | 727 | 159 | 15 |
| Filter B Scan 2 Day 3 | 14856 | 13911 | 755 | 177 | 13 |
| Filter B Scan 3 Day 3 | 14781 | 13839 | 748 | 179 | 15 |
| **Mean** | 14169 | 13268 | 730 | 160 | 11 |
| **Standard Deviation** | 400 | 375 | 17 | 11 | 3 |
| **RSD %** | 2.82 | 2.83 | 2.27 | 6.92 | 23.48 |

**Table 3:** Results of orientational independence tests where four repeat measurements were performed of the same filter with a 90 degree rotation between repeat analyses.*

| Sample Name | Total Particles | 2-10µm | 10-25µm | 25-100µm | >100µm |
|---|---|---|---|---|---|
| Filter B Orientation 0° | 13862 | 12981 | 714 | 157 | 10 |
| Filter B Orientation 90° | 13766 | 12915 | 700 | 143 | 8 |
| Filter B Orientation 180° | 13422 | 12536 | 728 | 153 | 5 |
| Filter B Orientation 270° | 13591 | 12724 | 709 | 151 | 7 |
| **Mean** | 13660 | 12789 | 713 | 151 | 8 |
| **Standard Deviation** | 194 | 201 | 12 | 6 | 2 |
| **RSD %** | 1.42 | 1.57 | 1.64 | 3.90 | 27.76 |

*independent customer results presented - analyses were not carried out in a controlled clean environment



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## Summary

Enumeration of Foreign Particulate Matter by microscopy based analysis is growing in many different industries and notably the pharmaceutical industry.

Manual analysis is time consuming and open to human subjectivity but the Malvern Morphologi G3 offers a solution which combines automated analysis of the filter sample with powerful software that allows FPM to be detected even down to 2 μm in size with a single scan using the 10 X objective in a short period of time, and to be automatically classified into specified size brackets.

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[1] Foreign Particles Testing in Orally Inhaled and Nasal Drug Products. J. Blanchard *et. al.* Pharmaceutical research, Vol 21, No 12, December 2004,

[2] Best Practices for Managing Quality and Safety of Foreign Particles in Orally Inhaled and Nasal Brig Products, and an Evaluation of Clinical Relevance. J. Blanchard *Et. al*. Pharmaceutical Research, Vol 24, No 3, March 2007.

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