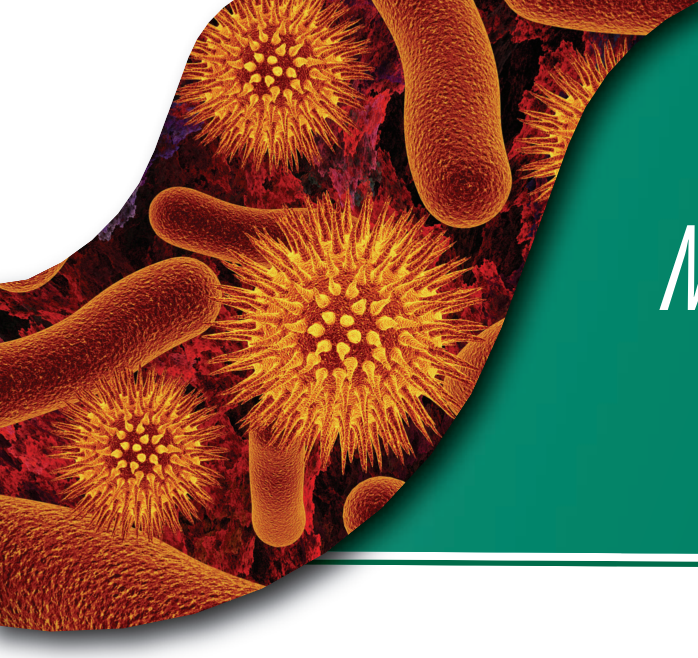

focus on

Microscopy & Microtechniques

Importance of Sample Dispersion in Microscopy-Based Particle Characterisation

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Automated image analysis offers high-resolution measurement of particle size and particle shape through direct image capture. It delivers highly valuable data for a wide range of particle sizes, typically from below 1 µm up to several thousand, and is ideal for many sample types.

While automated particle characterisation has clear advantages compared to laborious and time-consuming manual microscopy methods, harnessing the maximum benefit from this powerful tool requires appropriate sample preparation. Indeed, it constitutes the most critical step in the particle characterisation process since even highly advanced instrumentation cannot generate good results from poorly dispersed samples. Appropriate preparation methods are therefore needed for all sample types, whether dry powders, liquids or particles collected on filters.

Automation advantage

The advent of advanced automated image analysis has revolutionised particle characterisation, overcoming the many drawbacks associated with conventional manual microscopy. Automated systems can measure hundreds of thousands of particles in minutes, eliminate operator bias and human subjectivity, and through the use of Standard Operating Procedures (SOPs) enable complete transferability of analysis methods, from the research laboratory to the quality control department for instance. In addition, the high-resolution images obtained allow the calculation of detailed morphological data.

Optimising sample preparation and dispersion

As with many measurement techniques, the old adage of 'rubbish in, rubbish out' can be said to apply for automated image analysis. Therefore, when developing a new SOP for the analysis of a particular sample type, it is worth allocating the major proportion of the available time on fine-tuning the sample presentation and preparation steps, paying particular attention to sample dispersion and particle orientation.

As image analysis measures a two-dimensional projection of a particle, it is important that particles adopt a consistent orientation with the largest face presented towards the camera. This allows the detection of differences in particle morphology, in contrast to random orientation, which has the effect of averaging out valuable size and shape data. As well as meeting the requirements for consistent orientation, the goal of sample dispersion is to achieve a good separation of particles without inflicting any damage. Preparation methods for three sample types that are commonly analysed by microscopy-based systems are discussed below.

Dry powders

Reliable measurement of dry powders requires strict control of dispersion conditions. The ideal dispersion will contain discrete particles and have a constant particle density across the sample area. In addition, a consistent size distribution should be maintained, something that is especially important if the required area for imaging is a sub-region of the entire sample.

The powder dispersion method should be gentle enough to ensure primary particles are not broken and also, if the extent of agglomeration is of interest, to leave the agglomerates intact. Equally, if the main focus is primary particle size and shape then it may be preferable to break up agglomerates into their constituents. A sample preparation system that gives the user control over the intensity of the dispersal force is therefore ideal, enabling optimisation for different powders.

Toxicity is another important consideration and some dry powder dispersal methods can cause the release of particles into the atmosphere, especially where particle size is small. Therefore, if hazardous materials are being handled the dispersion system must minimise operator exposure.

Dispersion systems

There are many dedicated solutions available for dry powder dispersions that have the significant advantage of improving reproducibility and reducing sample preparation time. However, integrating the sample dispersion unit into the particle characterisation system significantly simplifies and streamlines the entire measurement process.

In the example shown in *Figure 1*, the dry powder is placed into the dispersion unit between two thin metal foils. An instantaneous pulse of compressed air is applied to break the foils (the ability to control the air pressure reduces the risk of particle damage), allowing the particles to accelerate into the main body of the chamber. The sudden expansion of air causes turbulence that disperses the sample onto a glass plate.

This system allows precise control over multiple variables including foil type, sample volume, injection pressure, injection time and settling time. Once optimum conditions are established, the dispersion parameters can be defined as part of the analysis SOP, enabling highly reproducible measurements. An additional advantage is that measurements are made in an enclosed sample carrier, minimising environmental exposure and reducing sample wastage.

In general, compressed air systems are preferable to vacuum dispersion methods since these can be too aggressive for fragile particles. Another common dispersion method involves driving a powder into a surface (a metal ball for example) to disperse the particles. While such collision-based methods are unsuitable for fragile particles, they may offer a useful approach for dealing with robust agglomerates that are difficult to separate.

Wet dispersion

Examining liquid samples with conventional microscopy instrumentation presents a challenge, the major obstacle being the limited volume that can be accommodated under a cover slip. This makes it difficult to obtain statistically relevant quantitative data for larger particles.

A novel solution is to use a large volume wet cell, such as that shown in *Figure 2*, which consists of two glass windows separated by one or more spacers, held together by a magnetic clamping assembly that both prevents sample evaporation during analysis and allows easy cleaning between measurements.

With a 2-8ml interior volume and a large scan area, this particular cell enables representative sampling of larger particles , greatly reducing the possibility of sampling error and ensuring the availability of a sufficiently high number of particles for analysis.

Filters

Enumerating and characterising foreign particulate matter (FPM) is important in many industries. In the automotive industry for example, the presence of particulate contamination is a major factor governing the lifetime and reliability of fluid systems, while pharmaceutical manufacturing is covered by strict regulations on the permissible levels of FPM in injectable drugs, inhalers and nasal sprays. Traditionally, FPM is collected on filters, the analysis of which has until recently been the preserve of manual light microscopy. Some automated image analysis systems can now make measurements on filters directly, bringing the benefits of automation to this application. However, there are a number of specific requirements for a system to be able to carry out this specialised task:

Maintenance of filter integrity

Keeping the filter stretched flat is an absolute requirement for automated analysis and relies on a robust filter holder. A useful development is the glassless carrier *(Figure 3)*, which eliminates any risk either of counting particles on the surface of the glass or crushing particles beneath it. The filter holder shown here allows the presentation of two filters, which can be analysed successively without manual intervention.

High-resolution operation

In the latest systems a combination of high quality microscope objectives and scientific grade cameras enables the analysis of particles down to 2µm on suitable filters. Images of particles at the lower end of this size range still contain enough pixels to be counted confidently as genuine particles.

Effective data analysis

If particles cross two or more frames, as is typical for fibres, the software must identify the frames which contain the particle image, 'stitch' the pieces of the particle together, and then extract the whole edge-stitched particle from the background. Another useful software feature is z-stacking, which allows images of each frame, taken at different focal points to be combined before the particles are separated from the background. Large particles remain in focus, while any small particles in the well of the filter paper are counted.

Composite image generation

The ability to view a composite image of the entire scanned area serves a number of purposes, including verification that there were no faults in the filter paper itself. A combination of composite image and scatter plot can also visualise the position of particles of particular interest on a filter. Additionally, it can help assess the quality of filtration, verifying that the particles are evenly distributed over the filter and not limited to the edge of the scanned wetted area.

Figure 1. An integrated sample dispersion unit streamlines measurements (Morphologi G3, Malvern Instruments)

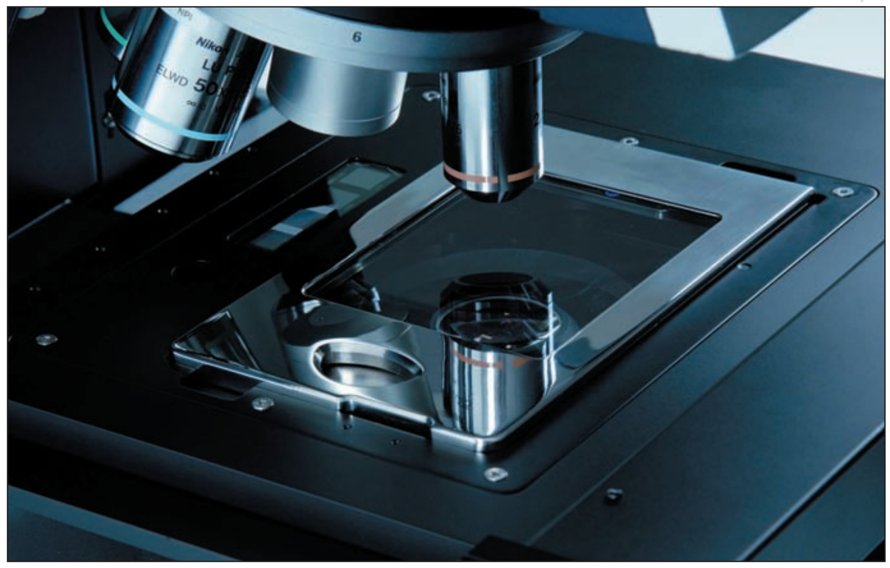

Figure 2. Large volume wet cell

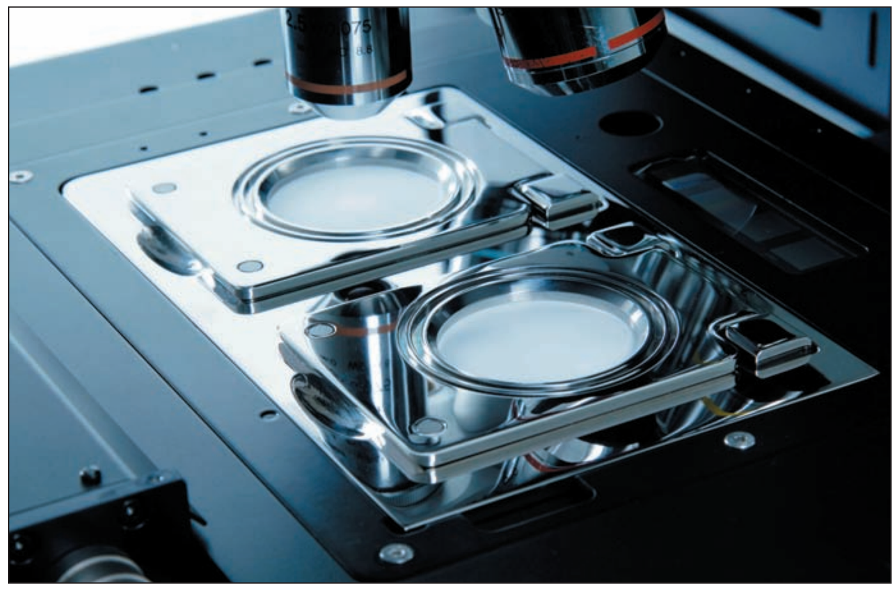

Figure 3. Glassless filter holder

Conclusion

Automated microscopy-based image analysis methods for particle characterisation deliver speed, objectivity and the acquisition of comprehensive morphological information. To maximise the reliability of the data, investing time in selecting the appropriate sample preparation and presentation method is undoubtedly worthwhile.

Dry powders require highly controllable dispersion conditions that depend largely on particle fragility and whether the main interest is primary particles or agglomerates; statistically reliable data from liquid samples necessitates the use of a sample holder that allows the measurement of a sufficiently large volume; and FPM analysis on filters requires an instrument with robust filter holders and software that can deal with the distinctive characteristics of this sample type. As well as delivering high quality data, the flexibility of microscopy-based image analysis systems such as the one exemplified here, which provide for the measurement of multiple sample types, are likely also to prove highly cost-effective.

Fast Scanning for the MultiMode Atomic Force Microscope

Bruker has announced new fast scanning capabilities for the MultiMode® 8 Atomic Force Microscope (AFM). The system's new ScanAsyst-HR feature provides a direct 6X increase in imaging rate for significantly improved research productivity. This remarkable development leverages Bruker's exclusive ScanAsyst® Imaging Mode, which has established itself as the industry standard for AFM ease of use. With the release of ScanAsyst-HR, Bruker becomes the first and only AFM manufacturer to provide fast scanning capabilities on two different AFM platforms, the MultiMode 8, which provides the highest resolution and research versatility and the Dimension FastScan, which provides the ultimate in AFM speed and large-sample AFM performance. Together, the MultiMode 8 and the Dimension FastScan make faster and more productive AFM a reality and make it accessible to researchers for almost any application and instrumentation budget.

"The MultiMode platform has been the gold standard for AFM resolution and versatility in the research community for over fifteen years," said Mark R.

Munch, PhD, President of the Bruker Nano Surfaces Division, said: "Adding ScanAsyst-HR makes the same excellent resolution and performance now available at scan rates 6X faster than conventional scan rates and enables up to 20X faster survey scanning, providing significant research advantages for the entire range of AFM applications."

David V. Rossi, Vice President and General Manager of Bruker's AFM Business, commented: "The MultiMode 8 builds on the celebrated reputation of the MultiMode platform as the gold standard for resolution and performance. ScanAsyst-HR is the latest in a long line of improvements to the MultiMode 8 capabilities, ease of use and productivity."

The MultiMode 8 is Bruker's premier high-resolution research AFM, which is available with an extensive list of new features and accessories that make it ideal for some of the most demanding AFM applications. ScanAsyst-HR on the MultiMode 8 makes faster AFM imaging accessible to an even broader segment of the AFM research market and additionally provides an upgrade path for existing users of older MultiMode AFMs.

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