Modern Particle Characterisation under the Microscope

The microscope retains a vital and wide-ranging role in modern industrial processes.

For instance, the pressure to optimise the quality of cast components requires a means of examining large, polished sections for defects both large and small whilst the identification of wear particles in lubricated systems is commonplace for many manufacturing companies. At the same time, continuing miniaturisation, higher packing densities and ever more rigorous quality assurance means that the physical size of particles capable of adversely affecting industrial processing and product quality is diminishing rapidly and making their characterisation more difficult.

And, many manufacturers need to microscopically examine their finished product to verify the absence of contaminating particles of all sizes, particularly in foodstuffs and pharmaceuticals. Indeed, many sectors, including pharmaceuticals and the automotive supply industry, need to document their complicity with guidelines and standards, such as CFR 21 part 11 and ISO 16232.

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Miniaturisation and increasingly rigorous quality standards mandate the use of analysis systems able to rapidly detect, measure and categorise increasingly small particles, of the order of a few micrometers, whilst still being able to cope with large particles or fragments. However, the data volumes involved in acquiring high-resolution images of these increasingly small particles within a large and heterogeneous sample field overwhelms the available memory in even high-specification computers and workstations.

Until now, the only answer has been to measure twice – at high resolution for small particles and, separately, at lower resolution for larger particles. A fast and accurate method to characterise the whole population of particles and their possible origins would bolster product confidence and boost company revenues, through decreased production downtime and quality assurance man-hours.

PARTICLE ANALYSER

Particle Analyser images complete samples in a single step, using either the SteREO Discovery range of stereomicroscopes (Carl Zeiss) for particles larger than 25 microns or the Axio Imager range of upright microscopes (Carl Zeiss) for particles down to 2.5 microns (*Figure 1*). The system measures whole heterogeneous populations of particles to allow automatic classification of particle types, and deliver fully resolved images on demand.

Figure 1. Carl Zeiss Particle Analyser delivers Increased Reproducibility for Automatic Quality Inspection

Both systems offer motorised polarisation and offer a rapid, one-step method of distinguishing between metallic and non-metallic particles using a specially integrated segmentation function. Until now, this was another procedure that required two scans of the sample – with polarised light being used to identify the particles and bright field used to determine the degree of reflection. The procedure is automatic and secure, and eliminates the need for manually applied corrections, with all the measurement parameters and system configuration stored with the results to guarantee consistent quality of measured results from batch-to-batch, regardless of a change in user.

Support for national and international norms, such as ISO 4406, ISO 16232, and VDA Vol. 19, is included and company-specific standards or demands outside the standards may also be implemented easily by the user. Also, the directives of the Food and Drug Administration (FDA) can be implemented for applications in the pharmaceutical industry and medical technology sector (*Table 1*).

Particle Analyser overcomes the problems of imaging large samples at high resolution in a single step by using the mosaic-image technique across the entire particle size range and combining that with an innovative approach to image storage and display.

Table 1. Conformity with International Standards

Several international standards call for the detection and characterisation of particulate contamination:

VDAS 19 (Germany) and ISO 16232 (International)
Road vehicles – cleanliness of components of fluid circuits

ISO 4406
Hydraulic fluid power – Fluids – Method for coding the level of contamination by solid particles

NAS 1638
Cleanliness requirements of parts used in Hydraulic Systems Aerospace Industries association – National Aerospace Standard

FDA's 21 CFR Part 11
Stipulates that all applications are validated conforming to GxP, with secure user-differentiated logons, log of all key events and data storage in secure, uneditable area.

MOSAIC IMAGING

In order to acquire images of the smallest particles of interest in any given sample, it is inevitable that only a small part of the total sample will be captured by the camera image and, therefore, multiple images will be required – possibly many hundreds. This is not a problem for a software-controlled, motorised microscope as the sample is automatically moved by the motorised stage, high resolution images are recorded at each position, and a mosaic image of the entire sample put together by combining all the partial images. Particle Analyser automatically uses the mosaic-image technique to accurately capture and measure all particles, whether a few microns or millimetres in scale. If the software detects that the reproducibility of the stage movement is too low for the particle size (for example, a higher number in microns than the pixel scaling), overlapping image acquisition with a software stitching step is automatically implemented. Additionally, the software can also implement Auto Focus between partial images.

This method of image capture results in data volumes up to the order of gigabytes. Until now, this would have limited image processing and measurement of mosaic-images to just one-image at a time. However, rather than holding the complete overall image in the computer's RAM, Particle Analyser holds a reduced-resolution form with only the relevant sub-region loaded for display on the monitor. This means that the computer's memory is only burdened with the current frame of interest, allowing the remainder to be utilised for analysis, with measurement performed on the fully resolved image.

CROSSING BOUNDARIES

The technique of memory management is not new, having already proven itself on the Internet. But, it has not been applied before to the processing and measurement of composite microscope images and means that it is now possible to carry out a full analysis on images that are larger than the available RAM.

In particular, particles that straddle the boundary between partial images can now be measured automatically in their entirety rather than in two or more sections and then added together manually (*Figure 2*). Importantly, users are assured that a single measurement process captures, displays and measures all particles. Moreover, the reproducibility of the image acquisition and evaluation process is guaranteed. A complete set of instrument parameters is stored with every image, including zoom setting (from a stereo microscope), objective magnification

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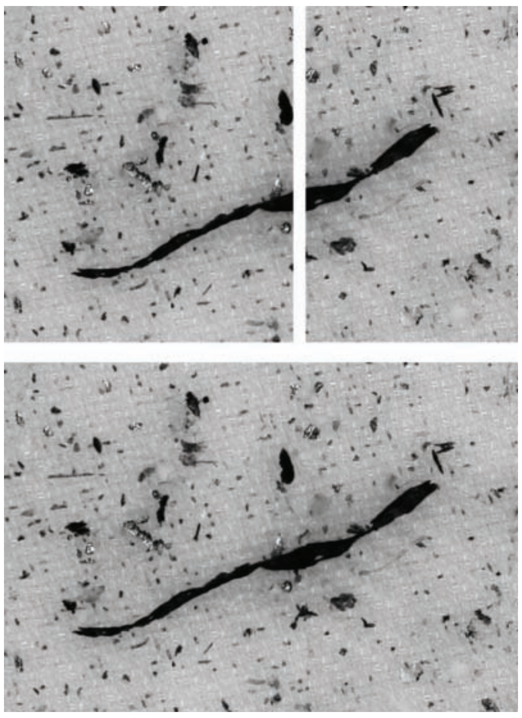

Figure 2. Particle cut off when acquiring single images

(from a compound-light microscope), illuminations setting, contrasting technique, scaling factors, auto-focus settings and camera settings. Should there be a subsequent need to re-analyse a sample, reactivation of all these parameters can be achieved without user intervention.

Similarly, this degree of automation provides non-subjective results with minimal user involvement for repetitive sample analysis. And, automated focus, calibration and diagnostic procedures ensure results accuracy and system stability.

No matter how complex the task, your tool should make it convenient. From entering the project data, acquiring and processing the image, measurement and data analysis, through to creating and archiving the data files (CSV) and customisable reports (PDF), the system is designed for speed and simplicity. And, authorised system administrators can quickly adapt the system to new test specifications and standards without needing external support and additional programming.

IN PRACTICE

Typical applications include:

Casting Defects
It is a common manufacturing process to die cast aluminium and magnesium components. Quality is fundamentally influenced by the casting and cooling processes where cavities and gas pores are difficult to avoid. In a bid to optimise the manufacturing process, many companies

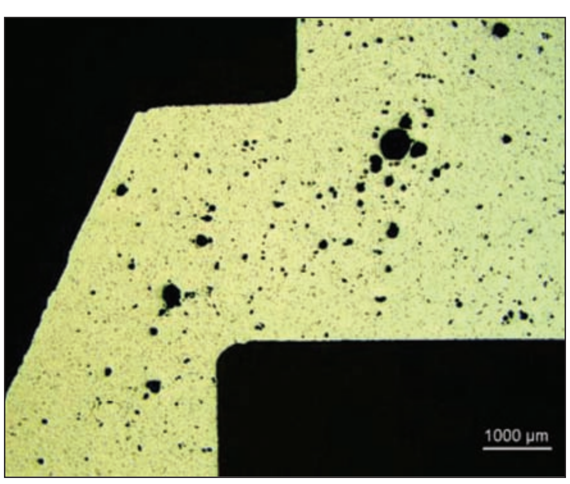

Figure 3. Structural defects in die cast components
Mosaic image of a total area of approx. $38mm^2$
Microscope: Axio Imager
Objective: EC Epiplan Neofluar 5x/0.13
Camera: AxioCam MRc

attempt to determine the area shares of fracture-prone casting defects and grade them according to size (*Figure 3*).

Once again, this can be achieved with a single scan with Particle Analyser without resorting to single image analysis.

Crystalline Forms in Ointment
Quality control of ointments may involve measuring the amount of active substance in crystalline form. For instance, fungicidal preparations for the skin and mucous membranes are tested for the suitable distribution of the active substance within the matrix material of the compound. Samples may be from new products in development or quality control samples of finished batches. To do this, ointment is smeared onto a slide and analysed under polarised light using 10X or 20X objectives, where the crystals of the active substance are clearly visible as dark structures and can be assessed using image analysis. To validate the system, a circular standard area of 100 square microns is measured every month and the result compared with a reference value.

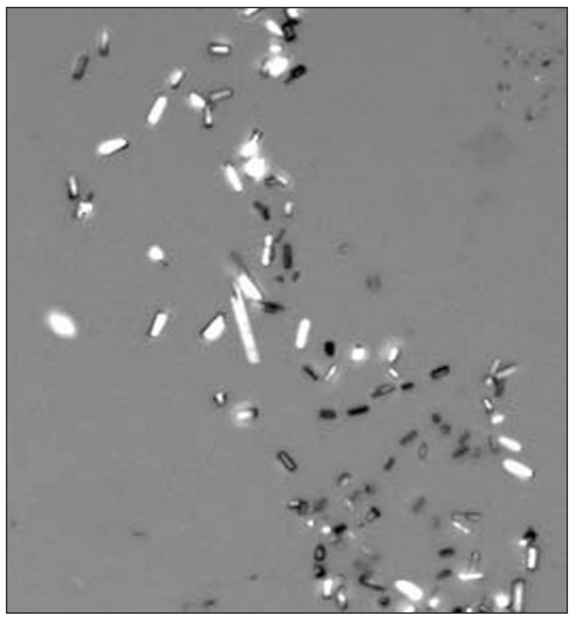

Figure 4. Active substance in crystalline form in an ointment sample
Microscope: Axio Imager
Objective: A-Plan 10x
Camera: AxioCam MRm

With Particle Analyser, large crystal agglomerates and small particles can be analysed in a single step and the distribution directly compared with the specifications held in the Standard Operating Process (SOP) automatically (*Figure 4*).

Plaque Analysis
Many vaccines and sera are tested by setting out a dilution series of the agents in a 24-well plate together with virus particles and cells. After incubation the wells are examined for the presence of plaques in the cell lawn caused by un-neutralised viruses (*Figure 5*).

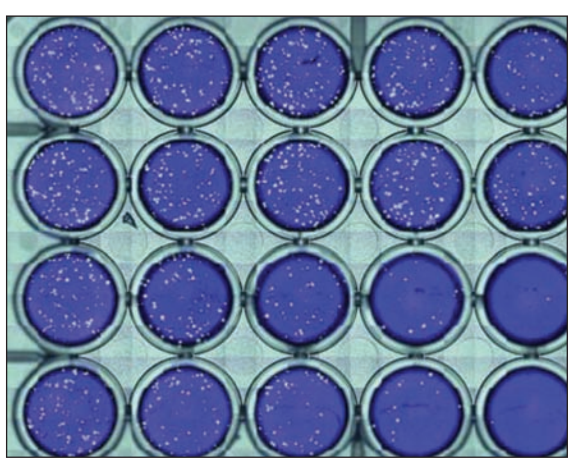

Figure 5. Viral plaques in 24-well plate
Microscope: SteREO Discovery.V12
Objective: Plan S 1.0x
Camera: AxioCam MRc

Using Particle Analyser, the entire plate can be scanned and the plaques automatically detected, separated and measured, with artefacts below a specified minimum size automatically excluded. The measured data and the original and result images of each plate are saved and, optionally, the entire process may be validated in conformity with GxP.

Residual Dirt on Filter Membranes
Many laboratories analyse particles trapped on filter discs. In the automotive industry, for instance, test components are rinsed with a defined amount of fluid, to wash off dirt or wear particles, which is then passed through the filter disc to capture the particles for analysis. The goal is to get a representative distribution of particles of a given type and, usually, the technician will check the result against defined maxima set out in International Standards (*Figure 6*).

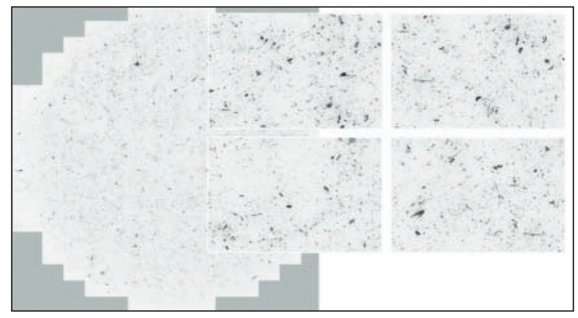

Figure 6. Residual dirt particles on filter membrane
Mosaic image consisting of over 200 single images
Microscope: Axio Imager
Objective: EC Epiplan Neofluar 5x/0.13
Camera: AxioCam MRc

With Particle Analyser users can, for the first time, scan their filter discs completely and contiguously, analyse the mosaic images to size and grade the particles and automatically report the results against the set standard. Particle Analyser will also automatically characterise particles, for instance to distinguish between reflecting and non-reflecting and reflecting details to identify metallic particles. Again, this data is obtained with a single scan of the sample. And, the software will also combine parameters, such as area, form factors, circumference and diameter, to size-grade large numbers of different particles.

As well as the automotive industry, this procedure is used in a very similar way to examine parental fluids in the pharmaceutical industry and at wind-energy plants to check the service intervals of lubricants.

CONCLUSION

The continuing integration of microscopy, digital camera technology and computing power opens up exciting new analysis tools to relieve the tedious and repetitive tasks involved in much of today's materials analysis procedures while profiting from the ability to meet future requirements. Above all, it is now possible to meet the seemingly contradictory requirements of characterising whole populations of particles while simultaneously recognising the smallest.