Leveraging Automated Microscopy Image Analysis for Particle Measurement

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Why Accurate Particle Measurements Are Critical

Successful manufacture and processing of powdered materials in pharmaceutical, print, paint and other industries, requires precise, detailed, and easily accessible data about particle shape and size. These particle attributes directly affect the quality of a product or the efficiency of an industrial process.

For example, particle size and shape directly affects: inhaler medications and their ability to flow to the lower lung; the stability of pharmaceutical creams; the quality of print toners; and the color, opacity and brightness of paints.

High standards in competitive markets alongside the mandates of regulatory organizations add to the pressure placed on particle manufacturers to provide statistically significant and reproducible particle size and shape data. This data is required by research and industry for the evaluation of micronized and nonmicronized particles for a variety of purposes.

Accurate and reproducible particle size and shape data are critical because they:

> Improve assessments of a particle-based product's or processes' quality
> Are necessary for evaluating lot-to-lot variation
> Must be provided for a product or process to be approved by regulatory organizations

Determining Particle Size and Shape

For several years, optical microscopy has been a reliable tool for observing and assessing particle shape and size. Due to the lack of automation and scalability of microscopy, it does not provide the means of analyzing tens of thousands of particles, and is consequently not a means by which statistically significant data can be generated.

Automated analysis of microscopy images has been leveraged more recently by the pharmaceutical industry to generate precisely this kind of statistically significant and reproducible data.

The advantages of automating microscopy are clear:

> A discrete sampling method aggregates multiple observations and compiles statistical results
> Data browsing tools facilitate data mining for validation purposes
> Alongside statistical information, image analysis provides visual information such as particle texture, color and shape
> Particles in excess of 2000 microns can be viewed using devices other than a microscope

Automated microscopy image analysis adds validity and value to data collected about particle shapes. Any product or process that hinges on particle attributes can benefit from this automated and scalable data generation technique.

Resolution and Accuracy

Image Analysis offers all the advantages known to high precision optics. With high precision optics, visible objects are quantifiable. Optical microscopy has been relied upon for many years to accurately evaluate shape for particles ranging in sizes from 1 micron to 2000 microns. The measurements taken on the image are detected as a number of pixels; a minimum of 100 pixels (10 pixels by 10 pixels) is required for detailed shape/texture evaluation, such as roughness and sphericity. The limit of resolution is determined by this objective. A high quality 100X optical objective (1.35 NA) gives a resolution of 0.1 micron. This resolution is in fact the minimum distance between two particles. In practice, since at least 100 pixels are required for shape analysis, 1 micron is the lower limit. However, smaller particles, down to 0.5 microns can be counted and measured in one direction accurately. For particle counting, the number of pixels representing each feature is less important, as long as it is detected, particles as small as 0.5 microns can be counted.

Shape Factors

Since the particles are deposited on a glass slide it is assumed that as they lay in their most stable position the largest plane is projected, as opposed to particles flowing through a chute where they fall randomly and are measured in any plane. Volume calculations can be applied if the habit of the particles is known. The Multi-Layer Grab algorithm allows the operator to reconstruct the image so that particles at different depths of focus (different sizes) can be measured accurately in the same run.

Statistical Significance

The measurements done on the particles will lead to important decisions concerning the process, the batch, etc. The operator must take the measurements so that the whole sample is statistically represented. For statistical purposes, one must perform an analysis of a minimum of 5000 particles from one sample. This is easily attained with Automated Image Analysis.

Importance of Sample Preparation

Sample preparation methods must be consistent. Yet, even with the strictest of protocols, preparation of glass slides is extremely delicate. To reduce the impact of slide to slide variability, it is better to sample a smaller number of particles across several slides than to measure the total number of particles on a single slide. The number of particles per slide will vary from slide to slide and also from one sample to another.

Attaining Statistical Significance Automatically

An automated system can reduce the analysis time significantly. For example, a conditional stop can prompt the system to terminate analysis on reaching a user-defined number of particles, or when the standard deviation stabilizes. All statistics can be recalculated in real time. Through automation and integration, it is possible to process large numbers of particles within minutes. Taking advantage of a fully automated system with data and user management capabilities guarantees repeatability and traceability of data and facilitates compliance with regulatory requirements such as CFR21 part 11.

Procedure

Step by step Image Analysis procedure

Image Acquisition:

The quality of the optical components and the resolution of the camera are of primary importance in the clarity of the image. Since meaningful results can only be obtained from representative images, proper sample preparation, adequate magnification and consistent illumination are critical.

Grey (or intensity-based) Image Enhancement:

Grey level algorithms are applied to define the edges of the features and increase the contrast between the phases. Many algorithms are available in current image analysis software. The art lies in combining these algorithms in the best way possible in order to accurately enhance and 'clean' the features of interest. A high contrast, noise free image is easy to threshold and requires fewer binary operations prior to measurements.

Binarization by Thresholding:

In this step, each pixel is assigned to a bitplane according to its grey level (in black and white images) or hue, saturation and intensity range (in color images), and is finally represented by a different color. The range of grey levels assigned to each color can be done automatically if the contrast between the grey level phases is sufficient. Ideally, the phases of interest correspond exactly to separate bitplanes, however some Binary Operation may be required.

Binary Operations:

At this stage, common features have been more or less successfully grouped into different colored bitplanes. The detection can be improved by using Binary Operations, these operations are applied to bitplanes to separate, delineate and classify features based on morphology or size, becoming representative of the phases or objects of interest.

Measurements:
When the particles of interest are well represented in the various bitplanes, measurements can be performed. Two types of measurements are used in particle characterization by automated image analysis: Object measurements provide information about the individual particles within a sample. Typical object measurements include length, aspect ratio, area, breadth, and so on. Relative measurements can determine the area percentage of either all the phases detected with respect to a reference phase (usually the total area of the selected phases). This type of measurement is cumulative for the total number of fields covered by the analysis.

Validation:
Modern Image Analysis software simplifies validation of sample measurements by using data browser tools. As long as the samples are still on the microscope platen, any field can be recalled. This gives the operator an opportunity to see oversize or undersize particles and if necessary, to reject artifacts, which may have been detected in the sample.

Results output:
With most modern Image Analysis Systems, results are compiled instantly and raw data can be exported or processed and displayed in a system-generated report.

Discussions/Conclusions

Although a discrete sampling method, Automated Image Analysis provides the user with access to valuable in formation on individual particles and data, which can then be validated using a data browser. Large numbers of particles can be measured rapidly: a typical analysis of 5000 to 10000 particles on four slides ranges from 3 minutes to 15 minutes depending on the size of the particles and the complexity of the analysis.

Carefully selected components, closely integrated (such as automated microscopes, high-precision motorized stages with auto-focus, high-resolution cameras and auto-calibration devices) allow for repeatability of measurements from analysis to analysis and from one instrument to another.

User and data management software make it easy to comply with regulatory standards such as 21CFR part 11. Fully integrated systems allow valuable walk-away time; expandable systems can be integrated with a robot, for an even higher throughput.