High Throughput **Size and Shape Analysis** of Micronized Powders

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Purpose

To establish the best possible procedure for obtaining detailed and precise morphological information on large numbers of micronized powders using image analysis.

Method

In order to be able to analyze fine powders under the microscope, several obstacles must be addressed. The first two obstacles are associated with sample preparation, and are particularly significant when analyzing a single slide. First, particles must be dispersed but not broken; secondly, it can be difficult to have enough single features on one slide to generate statistically significant results because of the presence of clusters.

The other common obstacle encountered in optical observation of micronized powders is the compromise which must be made between magnification and resolution. In most micronized samples, the focal plane of the finest particles is lower than the focal plane of the largest particles by a distance which is larger than the depth of field of the objective. This means that all the particles cannot be in focus simultaneously; therefore accuracy of measurement is compromised.

The sample preparation issues are addressed by processing more than one slide hence minimizing the slide to slide variability factor, and eliminating clusters by image algorithms. The focusing issue is addressed by software, using a multi-layer grab function which reconstructs an image from many focal planes, so that measurements are made on images where all the particles appear in focus.

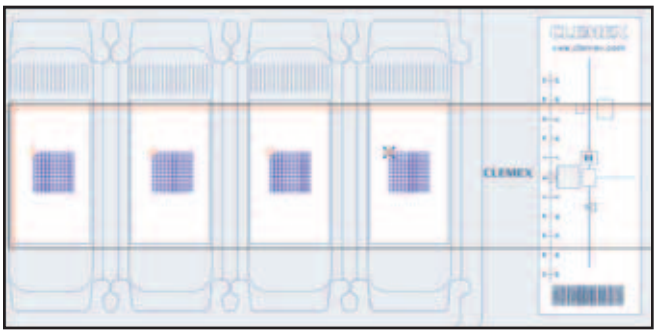
Procedure :

Four slides of micronized powder were prepared from one sample. Smaller amounts of the sample from four slides were analyzed rather than a large amount of the sample on one slide, to generate more statistically significant results.

A total of 5000 individual particles per slide were measured for Size, Shape and Volume by Image Analysis. Because of the wide size distribution, an image reconstruction step was used to produce images where all particles appear in focus, prior to binary image processing (Figs 1 to 3). Results were compiled per slide as well as combined for the whole sample after data validation.

Method (con't)

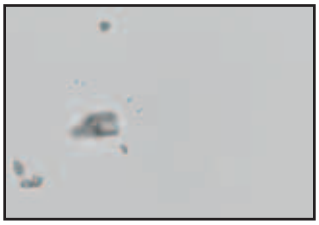
Stage pattern:



Equipment used :

- Fully Automated Optical Microscope with transmitted light
- N Plan 100X objective, 0.9 NA
- High Resolution Monochrome Camera
- Clemex Image Analysis Software

Figure 1. **Small Particles Detected**

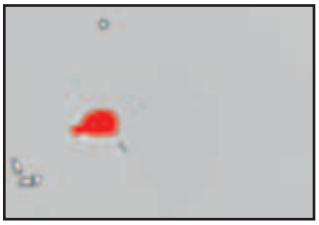


Micronized powder at 1000X. Small particles are in focus at lowest focal plane.

Micronized powder at 1000X. Grey Level Threshold on lowest focal plane.

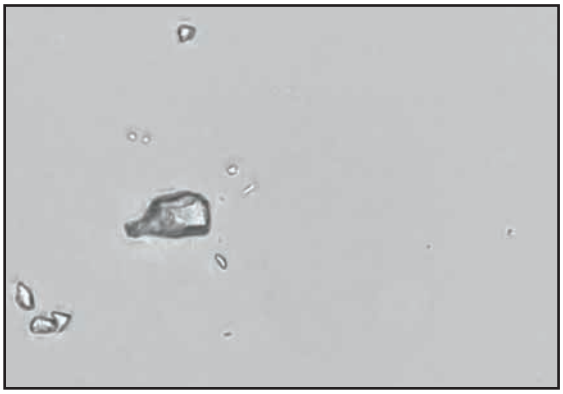
Figure 2. **Large Particles Detected**

Micronized powder at 1000X. Large particles are in focus at highest focal plane.



Micronized powder at 1000X. Grey Level Threshold on highest focal plane.

Figure 3. **Reconstructed Image**



Reconstructed image with large and small particles in focus.

Grey Threshold on reconstructed image. Large and small particles are in focus.

Conclusion

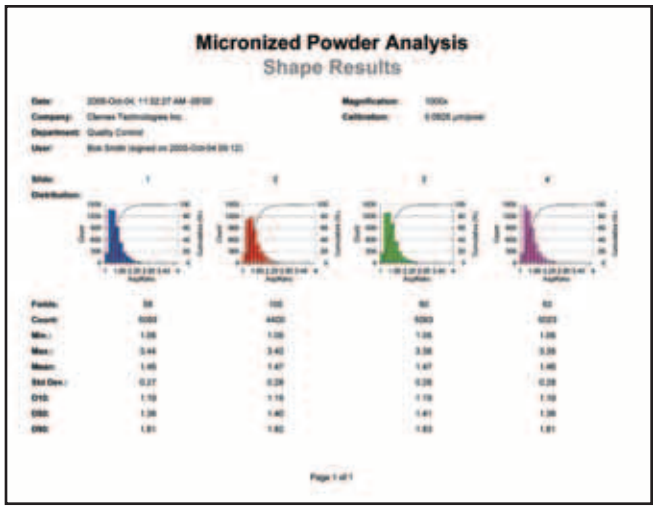
Analysis of micronized powders by Optical Microscopy offers the immense advantage that individual particles can be seen and measured. Critical factors such as sample preparation, microscope illumination and resolution and also camera resolution must be optimal in order to simply view the particles accurately.

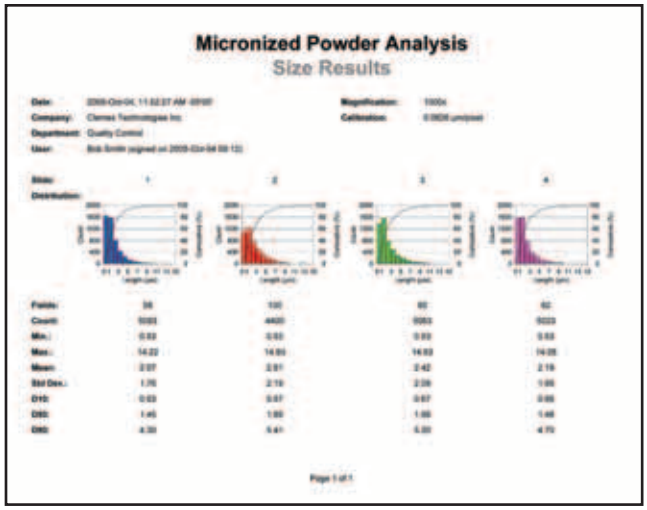
Once these conditions are met and a proper live image of the sample is achieved, powerful image analysis functions must be used in order to obtain a precise binary image of the objects to measure. This is crucial particularly at high magnifications such as 500X and 1000X and if the size distribution of the particles is wide because the largest and smallest particles lie in different focal planes.

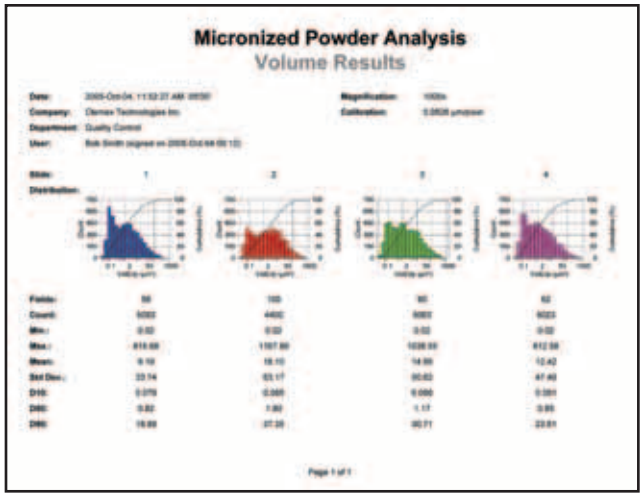
In such conditions a multi-layer image reconstruction is necessary; without it some objects would be missing depending on focus; small objects would be missing when focusing on the large ones and large objects would be unclear when focusing on the small ones; in both cases intermediate objects would have blurry edges.

Once accurate representation of the particles is achieved, relevant measures are performed on the individual objects and the results can be validated and artifacts removed (if any) prior to results output, since all the positions of the objects are remembered. This method allows for reproducibility and traceability of statistical results for detailed shape and size information of micronized powders.

Global results as well as results per slide were produced. The results show a large concentration of particles between 0.5 and 2.0 microns with particles as large as 14.93 microns.







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Results

Shape factor, size and volume % measurements were performed on 10,000 to 20,000 individual features. Because image analysis is a discrete sampling method, measurements for each particle are stored and each one can be validated by using a data browser. Images of peculiar individuals can also be stored for further reference.

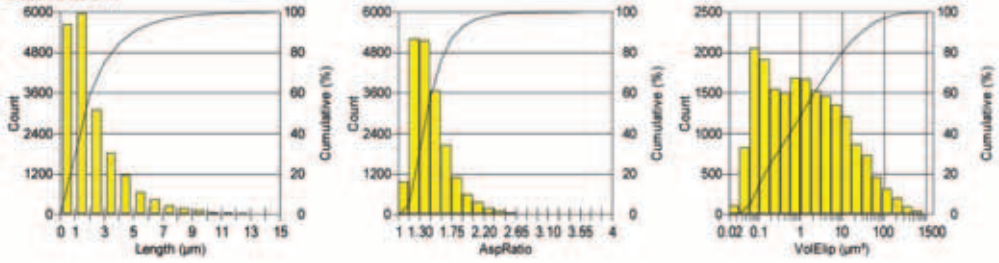
Micronized Powder Analysis

Global Results

Date: 2005-Oct-04, 11:52:27 AM -05'00'
Company: Clemex Technologies Inc.
Department: Quality Control
User: Bob Smith (signed on 2005-Oct-04 09:12)
Sample ID: 1, 2, 3 and 4

Magnification: 1000x
Calibration: 0.0826 µm/pixel
# of Fields: 310
# of Particles: 19578

Distribution:



	Length	Aspect Ratio	Volume
Min.:	0.53	1.05	0.02
Max.:	14.93	3.43	1167.80
Mean:	2.31	1.46	13.51
Std Dev.:	2.01	0.28	49.42
D10:	0.66	1.19	0.08
D50:	1.60	1.39	1.08
D90:	4.91	1.82	27.08

Comment: Global statistics of typical samples.

Approved by:

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