Developing a New High Definition Standard for Image Analysis

> ***"Image analysers have become so powerful that it is now possible to count several million particles..."***

Highest Resolution

One of the biggest beneficiaries of the parallel development of high power computers and digital cameras has been particle metrology. The resultant image analysis technique is now one of the highest resolution methods available today.

With an ever-increasing range of instruments having prices from $10,000 to over $100,000, end users are seeking an independent verification that they are selecting the right instrument for their application.

For this reason, there has been a significant interest in particle size calibration standards, in particular specific standards to exploit the high-resolution power and broad dynamic range of the technique.

Although image analysis can be applied down to submicron sizes, it is in the larger size range, typically over 1mm that the method is gaining in popularity as this represents the upper limit of many other techniques.

Precision Subdivision

Unlike single size latex standards where no special sample preparation is required, wide range or 'Polydisperse' standards must be prepared in such a way that each sample from the Master-batch is identical.

To determine both the resolution and dynamic range of image analysers, a new 'Multimodal' glass bead reference standard has been prepared having eight individual peaks from 500 to 2000 microns. However, such a standard represents a severe challenge to the subdivision process.

Not only must the overall percentile values be repeatable, but each of the eight peaks must be correctly assigned. A proprietary spinning riffler capable of accurately splitting into 100 bottles has been developed for the purpose *(Figure 1).*

Figure 1. A 100 stage spinning riffler for precision sample splitting.

A Multimodal Standard

Unlike conventional standards with Gaussian distributions, where only cumulative percentiles at 10%, 50% and 90% are reported, this standard not only covers percentiles from 5 – 95% but a value is assigned to each of the peaks (Figure 2).

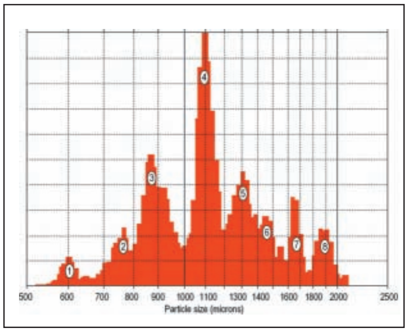

Figure 2. Individual peaks in a multimodal image analysis standard.

Over 30,000 microspheres were analysed using a NIST traceable reference microscope. To ensure the peaks were assigned to the correct values, the standard was fractionated and the separated peaks analysed independently *(Table 1).*

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Table 1. Comparison of fractionated peaks with those from the complete 500 – 2000 micron multistandard.

Peak	Individual fractions (µm)	Sizes from certificate (µm)
#1	601	592
#2	756	758
#3	857	858
#4	1108	1098
#5	1295	1301
#6	1447	1449
#7	1647	1640
#8	1865	1884

Finally, a precision electroformed sieve analysis was performed to ensure the cumulative distribution overlaid that obtained from the microscope.

Certification Process

One of the disadvantages of using a simple laboratory microscope for particle size analysis is the time taken for a measurement, not only from the point of view of collecting and analysing the data but the mechanics of preparing and analysing the slides.

The 500 – 2000 micron multimodal standard is supplied in 20g bottles. To perform the certification, the standard was subdivided into 1gram subsamples using the spinning riffler. Data from each sub-sample were then combined until a statistically robust number of particles were counted, in this case 33,000.

Test Case – Repeatability

The first and most important question to be asked of a polydisperse standard is ‘How does each bottle of standard compare?’ This question was answered using a Haver CPA image analyser. When 10 x 20g bottles containing approximately 16,000 beads were analysed, the results were identical *(Figure 3).*

Test Case – Particle Count

Image analysers have become so powerful that it is now possible to count several million particles, but is that really necessary? In the case of this standard that would represent over 10kg of material.

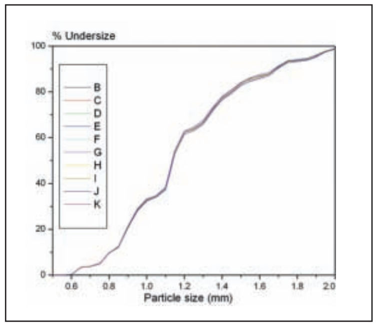

Figure 3. Repeatability measurement on 10 x 20g samples (Haver CPA).

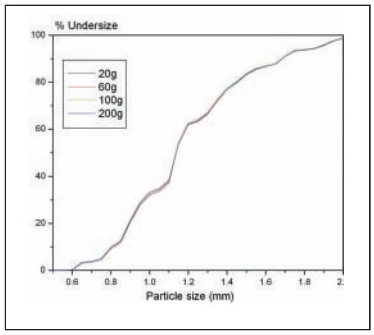

Figure 4. Effect of weight on repeatability (Haver CPA).

To determine the optimal particle count, the Haver CPA was used to compare the results from 20g, 60g, 100g and 200g of the standard. Again the results were identical (Figure 4). This means that a 20g bottle of the standard is quite sufficient to obtain statistically robust data.

This last dataset also illustrates the speed of analysis that can be achieved with the latest automated image analysis instruments. The time required for the certification of the 500 – 2000 micron multimodal standard of 8 hours was reduced to less than 4 minutes for the same particle count.

Test Case – Resolution

Having established the optimum weight of the standard and the repeatability of measurement, the final analysis is the ability of an image analyser to correctly position all the eight peaks. The results in *Table 2* show that, in the case of the Haver CPA, there is excellent agreement with the certified values.

Conclusion

The availability of an independent and challenging particle size reference standard for image analysis now enables the prospective purchaser to unequivocally assess the performance of this new class of particle size analyser.

High-speed electronics both in the computer and camera have reduced analysis times using conventional microscopy from several hours, or even days, down to a few minutes. The debate on the particle numbers then becomes largely irrelevant as 50,000 particles can be counted in only about 5 minutes. However, it has been shown that about 16,000 particles are quite sufficient to give excellent repeatability, even at the extreme ends of the size distribution.

One of the biggest advantages of image analysis is the ability to identify and size closely lying peaks in a sample. This new standard now enables the resolution to be quantified and ensures that accuracy is maintained across the dynamic range of the instrument.

Table 2. Performance of Haver CPA in analysing the individual peaks in a 500 – 2000µm image analysis standard (multistandard).

Peak #	1	2	3	4	5	6	7	8
Certificate (µm)	592	758	858	1098	1301	1449	1640	1884
Haver CPA (µm)	610	750	860	1100	1300	1400	1650	1860

**209.6 g or 153,420 particles*

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