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Errors in Optical Microscope Measurements using Image Analysis

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Particle size and shape analysis using optical microscopy and digital image analysis (OM/IA) is increasingly being used in the development of pharmaceutical products. These methods can be utilized either for comparison with other size techniques, such as laser diffraction, or as primary measurement techniques. In either case, it is important to understand and to quantify the accuracy and precision of such methods.

The accuracy of OM/IA will be limited by the following: 1) the resolution of the optics of the microscope; 2) the resolution of camera and digitization of the resulting image; and 3) the measurement algorithm of the image analysis software [1,2]. The accuracy is affected by the method of calibration along as well as the accuracy of the micrometer used for the primary calibration. The accuracy can be tested using well-characterized standards such as NIST SRM 1965 which is a microscope slide with embedded polystyrene spheres with a measured diameter of 9.89 ± 0.04 µm [3]. Using a Nikon Optiphot microscope, a Clemex micrometer for calibration and the Clemex Vision image analysis system, the following mean values were obtained: 10x (0.5 N.A.) 10.6 ± 1.0 µm, 20x (0.75 N.A.) 10.1 ± 0.73 µm,. These results indicate that such systems can achieve reasonably good accuracy.

There are many more factors that must be considered with regard to the precision of OM/IA methods. The factors can be simplified by separating them into two categories of (1) instrumental errors and (2) sampling errors. In brief, errors due to sampling are considerably larger than those due to instrumental factors. SRM 1965 was used to investigate the instrumental errors in two sets of tests. One field of view (FOV) was chosen and in the first set of tests one collected image was run through the measurement routine 10 times. There were no differences in results . The same FOV was recollected 10 times and then the particles measured using the same measurement routine for each image. There was some variability but it was small (Table 1). For instance, the mean circular diameter was 27.2 µm with a standard deviation of 0.2 µm. This leads to 95% confidence limits of ± 0.14 µm and, consequently, an indication that instrumental error is small.

There is a long list of possible errors that can be introduced from sampling, but the subject can be narrowed considerably by simply looking at the laboratory sample and assuming it is representative of the batch. The key issue is how many particles must be measured from how many FOV's from how many independent preparations (slides) to yield a good estimate of the desired parameters such as mean particle diameter. Errors in sampling were assessed using a glass particle standard from Duke Scientific Corporation (Palo Alto, CA, USA) with a mean particle size of 20.3 µm and a standard deviation of 2.1 µm. For this study, 10 slides were prepared and 50 FOV's were evaluated for each slide (Table 1). The mean diameter and D50 (median) can be accurately estimated with just a few slides whereas the D10, D90, and Maximum require more (Fig. 1).

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Table 1 Estimates of Instrumental (SRM 1965) and Sampling (Duke Standard) Errors. Sampling errors are typically much larger than instrumental ones. Std Dev – Standard Deviation; C.V. - Coefficient of Variation

Parameter	Minimum	Maximum	Mean	Std Dev	Count	D10	D50	D90
SRM 1965, 1 FOV, 10 Image Collections and Measurements								
Mean	2.9	72.1	27.2	12.1	61	15.5	24.8	41.2
Std Dev	0.8	0.1	0.2	0.2	0	0.2	0.2	0.3
C.V. (%)	27.6	0.1	0.7	1.6	-	1.3	0.8	0.7
Duke Glass Standard, 10 Independent Preparations, 50 FOV's								
Mean	4.5	63.3	22.9	6.5	2421	15.1	23.1	27.3
Std Dev	0.4	11.7	1.4	0.6	371	4.0	0.4	2.5
C.V. (%)	8.9	18.5	6.1	9.2	-	26.5	1.7	9.2

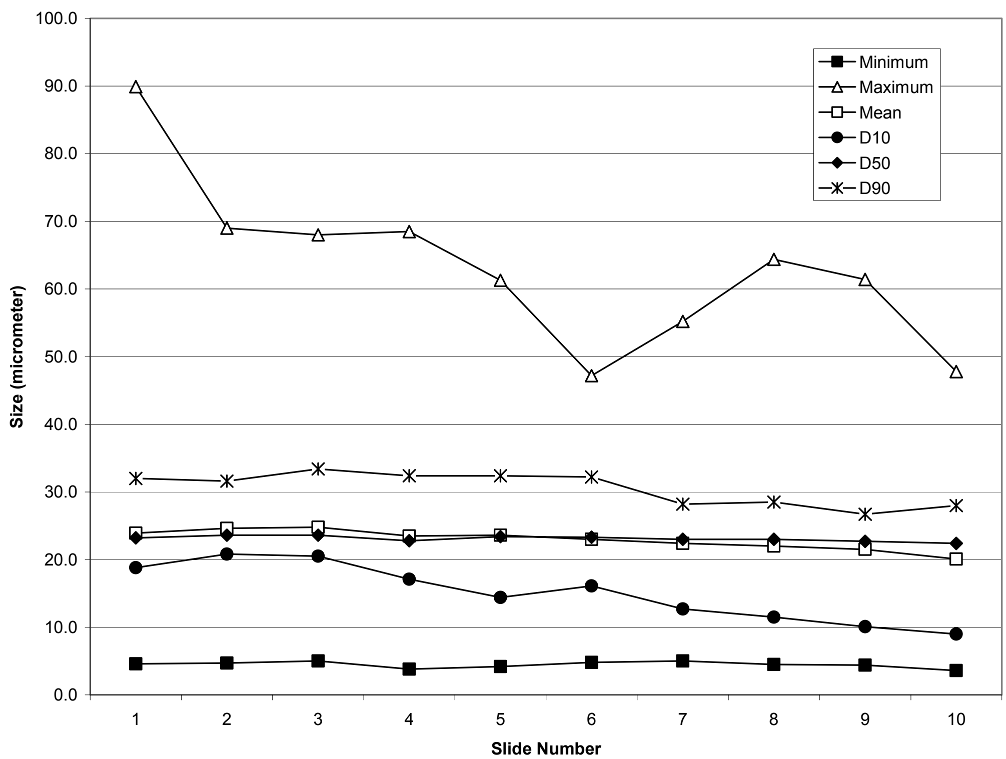

FIG. 1. Circular Diameter Parameters versus Slide Number. Illustrates that the mean and D50 (median) can be estimated with just a few slides, whereas D10, D90, and Maximum require more.

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