

Sampling 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. This paper is the second in a series examining errors in OM/IA [1].

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 [2, 3]. The accuracy is affected by the method of calibration and 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 $9.89 \pm 0.04 \mu\text{m}$ [4] and other standards such as a glass particle standard from Duke Scientific Corporation (Palo Alto, CA, USA) with a mean particle size of $20.3 \mu\text{m}$ and a standard deviation of $2.1 \mu\text{m}$. Tables 1 and 2 present results using a Leica microscope (infinity corrected optics), a Clemex micrometer for calibration and the Clemex Vision image analysis system. These results indicate that such systems can achieve good accuracy.

Previous tests indicated that the measurement precision is dominated by sampling effects [1]. It was suggested that, for real particles with irregular shapes, it is better to measure fewer fields of view (FOV) on more slides in order to improve precision. Further study casts some doubt on that suggestion. The results of Table 2 indicate that slide-to-slide variability is small compared to the within-slide variability. In other words, the results from one slide are not appreciably different from the mean of many slides. Consequently, testing more particles on one slide is probably a good strategy for this sample. Is that true for irregularly-shaped particles with a non-Gaussian distribution?

Tables 3 and 4 present the results of measurements of pharmaceutical compound A which has a non-Gaussian particle size distribution and irregularly shaped particles. For the first set of tests, one slide was prepared and 5 different regions of the slide were selected. For each region, 25 FOV were selected and the particles measured. Clearly, there is a high region-to-region variability. In the second set of tests, 4 slides were prepared and one region with 25 FOV were measured for each. Interestingly, the slide-to-slide variability was less than that of the region-to-region variability.

These results indicate that the best measurement strategy may be to test multiple regions on a limited number of slides. In any case, this sort of sampling analysis should be conducted for any material for which an OM/IA particle size method is being developed.

REFERENCES

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Table 1 Accuracy and Resolution, SRM1965, Nominal Diameter = $9.89 \pm 0.04 \mu\text{m}$

Objective	Mean Diameter (μm)	Numerical Aperture	Resolution Limit (μm)
10x	10.6 ± 1.0	0.30	1.1
20x	10.1 ± 0.7	0.50	0.7
30x	9.8 ± 0.6	0.75	0.5

Table 2 Precision using Duke Scientific glass spheres, Nominal diameter = $20.3 \pm 1.4 \mu\text{m}$

Slide	Mean Diameter (μm)	Standard Dev. (μm)	Population
1	21.3	1.71	814
2	21.0	1.71	1233
3	20.7	1.71	1237
4	21.2	1.72	530
Mean	21.0	1.71	3814

Table 3 Within Slide Variability, 5 Regions of Slide, 25 fields of view each, Compound A

Region	Population	Mean (μm)	x50	x90	Max. (μm)
1	3345	10.1	8.7	16.8	179
2	3076	11.9	9.6	20.2	142
3	1595	14.1	10.6	27.1	92
4	2212	9.6	7.8	14.7	89
5	5572	12.8	10.1	23.0	137
Mean	3160	11.7	9.4	20.6	128

Table 4 Slide to Slide Variability, 4 Slides, 25 fields of view each, Compound A

Slide	Population	Mean (μm)	x50	x90	Max. (μm)
1	3621	12.4	12.4	23.4	130
2	5572	12.8	10.1	23.0	137
3	3444	10.2	8.2	16.9	66
4	4448	12.4	9.2	21.7	111
Mean	4271	12.0	10.0	21.3	111