PROBLEMS IN PARTICLE SIZE: LASER DIFFRACTION OBSERVATIONS

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KEY POINTS

The following key points are discussed:

- It is important that the results generated by a particle size analysis are thoroughly understood in the context of their appropriate application.
- The identification and correction of problems encountered in laser diffraction analysis is discussed.
- Examining the sample under a microscope is a critical step in verifying that the results obtained by a particle size analysis correspond well to the observed particle size.
- Broken particles may occur due to use of excessive ultrasonic energy in liquid sample preparation or excessive air pressure in dry powder sample preparation.
- Most homogenous solid particle sample types should produce a continuous particle size distribution, so the occurrence of a distinct disconnected or semi-disconnected peak should be considered suspicious and thoroughly investigated.
- Distributions with distinctly different modes of occurrence (disconnected peaks) can indicate issues such as bubble peaks, thermal artifacts, dry dispersion artifacts, opalescent or reflective artifacts, and optical model artifacts.
- Orthogonal techniques are useful in verifying the accuracy of a particle size distribution.
- When the particle size distribution of a material needs to be measured, steps should be taken to ensure that the measured data output reflects the test sample rather than an artifact, bubble, or other source of data contamination.

INTRODUCTION

The results obtained by a particle size analysis can easily be taken for granted. If the particle size distribution of a material must be quantified and reported, it is important that the full implications of the analytical technique and generated data are investigated and understood. While complying with designated testing methods and procedures is of great importance in a quality assurance environment, it can be argued that the purpose for testing is irrelevant if the results obtained by these methods are inaccurate. Using a consistent method is necessary for highlighting lot-to-lot variability, but the results only reflect the actual particle size insofar as they are known to be accurate. Classifying the "true" particle size of a material can be a difficult task, as the results obtained by an analysis are highly dependent on the technique by which they are measured. Even so, there are ways that a given particle size result can be verified to ensure greater confidence in the accuracy of the data.

When reviewing the results of a particle size analysis, there are several common red flags that can lead one to question the accuracy of a given data output. While many of these problems can apply to a variety of particle sizing methodologies, this article focuses on those common to laser diffraction (or laser light scattering), as it represents one of the most widely used techniques. This article aims to briefly discuss a few of these problems, how to identify them, and how to correct them.

BROKEN PARTICLES

While specific circumstances may necessitate otherwise for certain applications, the goal of a typical particle size analysis is to measure the particle size distribution of the individual or primary particles in a given sample. According to the National Institute of Standards and Testing (NIST), a primary particle is defined to be the smallest identifiable subdivision in a particulate system (1). It is important that the technique used to measure particle size does not itself result in altering the primary particle size. While this sounds simple enough, it can at times represent a gray area. The method used to disperse particulate must be examined closely. Observing the sample under a microscope prior to analysis is a critical step in verifying that the results obtained by a test correspond well to the expected particle size in a given dispersion.

Ultrasonic energy can be invaluable in dispersing agglomerated primary particulate in a liquid suspension. However, applying excessive ultrasonic energy can cause primary particulate to fracture in some cases. Particles especially susceptible to fracturing can include those which are elongated or acicular, soft or friable, or those that are platy. It is critical to observe the dispersion under a microscope before and after ultrasonic energy is applied in order to evaluate the effect of sonication and the progress of the dispersion.

Figure 1 shows the result of typical levels of ultrasonic energy on a particle type that is susceptible to fracturing. The photo on the left shows a hand-mixed dispersion of particulate up to approximately 250 microns in length. When ultrasonic energy is applied, as in the photo on the right, the particulate is shattered to less than 100 microns in length. The dispersion quality is otherwise sound. So without examination prior to the application of ultrasonic energy, it would be easy to unknowingly deliver significantly undersized data if the particles on the right were to be analyzed.
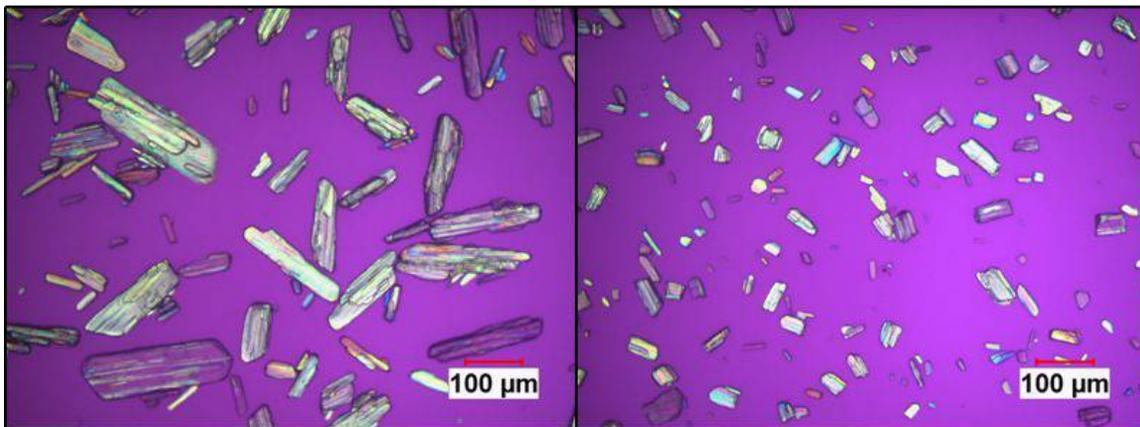

FIGURE 1. Particles in a liquid dispersion: hand-mixed (left) and with ultrasonic energy applied via ultrasonic bath (right).

Likewise, the effects of air pressure as a dispersion method for dry powders must be monitored carefully. Ideally, the pressure chosen must be sufficient to fully disperse primary particulate, while leaving these individual (primary) particles intact. However, because these processes happen simultaneously, the goal is to maximize dispersion while minimizing attrition. When a dry dispersion is necessary, it is useful and recommended to verify the quality of the results using a liquid dispersion technique. A liquid dispersion can

be directly observed under a microscope in its final state of dispersion. When multiple measurements are taken during the analysis, any instability in the preparation can be monitored. Many laser diffraction instruments can be outfitted to employ both wet and dry dispersion techniques, and this is ideal for comparison purposes. If possible, the dry dispersion pressure should be adjusted in order to best match the wet dispersion results, as the wet dispersion results can be directly verified to be accurate by microscopy.

Figure 2 illustrates a sample under several air pressure levels, each represented by a different colored line. This is referred to as a "pressure titration" in the industry. Note the overall decrease in particle size with increase in air pressure. The peak or mode (most frequently occurring size) shifts from about 500µm to about 300µm, while the lower end tail grows, indicating an increase in fines. This suggests that the particulate is breaking due to the dispersion pressure. Comparing these results to a liquid dispersion distribution (which is confirmed by microscopy) is an important step in verifying the appropriate air pressure for a proper dry dispersion. Figure 3 shows an overlay of a dry dispersion pressure level that corresponds well to the liquid dispersion distribution. Figure 4 is a photograph of the sample particulate as observed by microscopy.
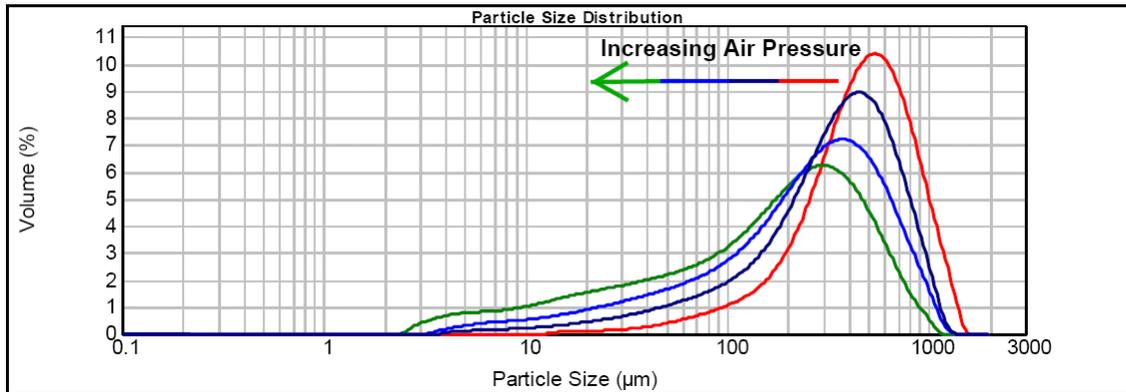

FIGURE 2. Typical particle size reduction with increasing air pressure. Red line = low air pressure, green line = high air pressure.
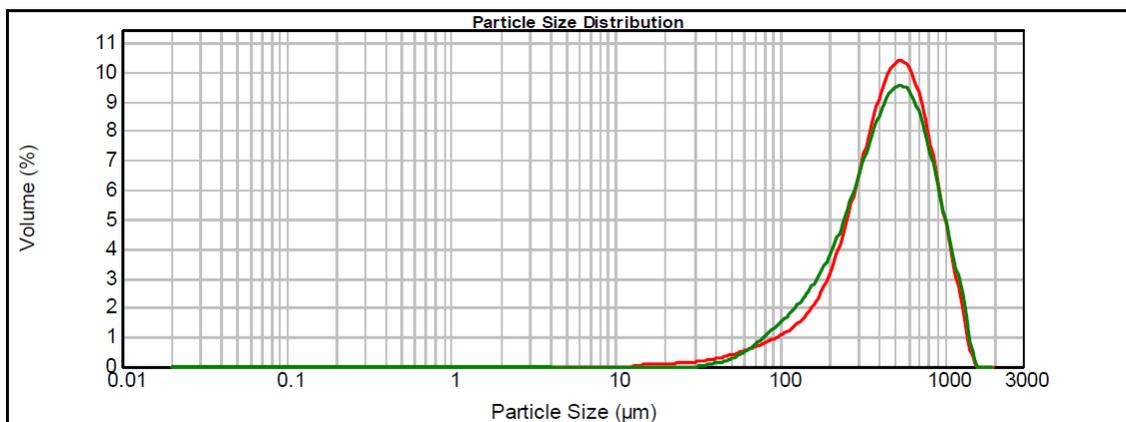

FIGURE 3. Overlay of a dry dispersion (lowest air pressure from Figure 2, red line) with a liquid dispersion (green line) of the same sample.

FIGURE 4. Photograph of particles in Figure 2 and Figure 3.

Dispersion techniques that alter the primary particle size of a material (intentionally or otherwise) can be problematic because they not only affect the results of a single measurement, but they can also consistently affect the results of repeated measurements. For this reason, it is imperative to verify the quality and accuracy of a dispersion at the time of method inception and validation. The repeatability and reproducibility of a method do not necessarily reflect the accuracy of the measurement. Without thoroughly establishing accuracy, the method risks producing repeatable yet erroneous data.

DISCONNECTED PEAKS
If little is known about the particle size distribution of a material submitted for testing, the assessment and interpretation of the data can be daunting for those less familiar with the field of particle characterization. However, there are a few rules of thumb that can be useful when reviewing the data. If the sample submitted for testing consists of a single species (such as the neat API) from a single batch or lot of material that has been processed in a uniform manner (no mixing of milled and unmilled portions), it generally can be assumed that the particle size distribution will be continuous. Naturally occurring materials tend to conform to a lognormal or skewed Gaussian distribution function. See Wiebull's paper for a discussion on a general statistical distribution function that can be applied to particle size distributions, as well as other examples (2). Depending on the particle shape, size, and collective nature (whether there is a presence of fused agglomerates and unfused primary particulate), the resulting distribution may be more or less broad with the possibility of having multiple minor modes. However, if these modes or peaks are disconnected from the primary distribution, the data should be thoroughly investigated to verify the credibility of the results.

While laser diffraction is an excellent tool for assessing lot-to-lot variability, the detection technique that makes it so useful in characterizing particle distribution can produce artificial components in the distribution if left unchecked. These can be easy to identify with experience. Examples of bubble peaks, thermal

artifacts, dry dispersion artifacts, opalescent/reflective particle artifacts, and optical model artifacts are discussed in the following sections.

BUBBLE PEAKS

Bubble peaks are one of the easiest problematic peaks to conceptualize. When a solid particle sample type is submitted for liquid dispersion analysis, it can be generally assumed that any bubbles or gaseous occurrence in the dispersion are not meant to be taken as part of the intended sample for analysis. Generally speaking, most particle size instruments and technologies cannot distinguish between species present in a distribution. Therefore, if signals are collected from both the sample and bubbles, the instrument will report both indiscriminately. While the operator of the instrument should be trained to identify and minimize a bubble problem before collecting data, it is useful to understand the basic identification features of such a problem should the results be taken under review.

In laser light scattering instruments, air bubble peaks can occur at 40-500 microns in water (3), and most commonly around 100-300 microns in this author's experience. Depending on the viscosity and surface tension of the dispersing medium, this size range can vary. Figure 5 is a typical example of a bubble peak occurring alongside a particle distribution in an aqueous dispersion. The bubble peak occurs at the coarse end at approximately 200µm in this example. There is a clear demarcation between the particle peak and the bubble peak. Though the bubble peak does not overlap with the sample particle size distribution peak, it will clearly influence the cumulative size moments and will render any associated data erroneous.
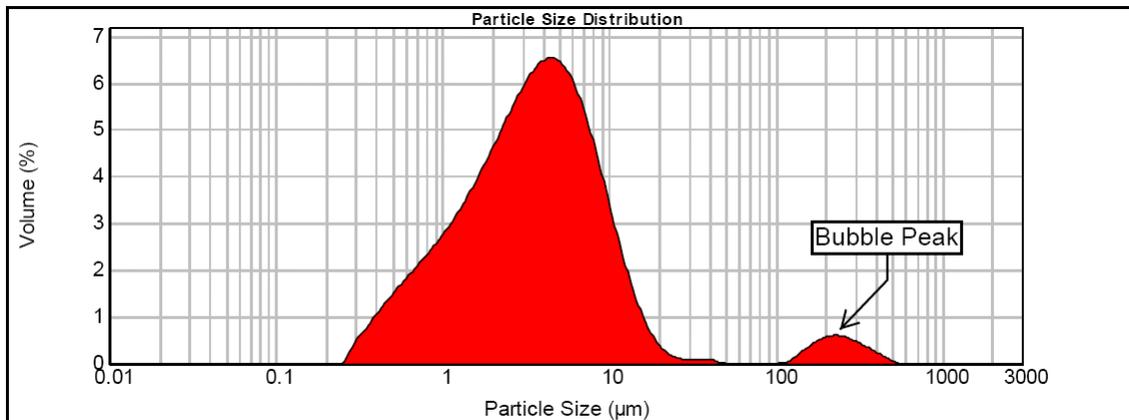

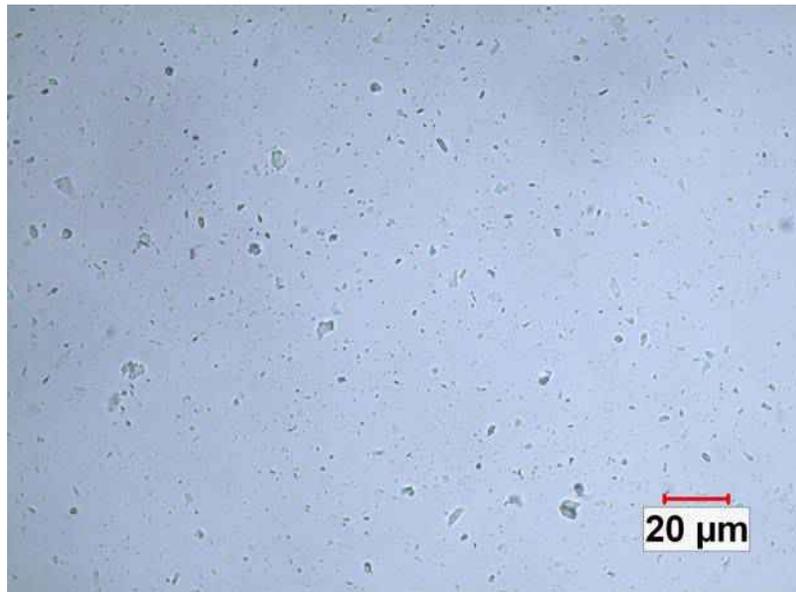**FIGURE 5**. Bubble peak in an aqueous dispersion.

FIGURE 6. Photograph of particles from Figure 5. Note the absence of particles in the 100µm-500µm range as compared to the distribution in Figure 5.

Even when all necessary precautions are taken, bubble peaks can occasionally appear in liquid dispersion data. It is important to be able to identify these peaks as distinct from the particulate to be measured, and to thereby invalidate them in a regulated environment. A simple check under the microscope is often sufficient to rule out the presence of particulate in the size range of the suspected bubble peak. Note that the particulate imaged in Figure 6 is conspicuously absent of any material greater than 100µm in size, unlike what is implied by the distribution in Figure 5. While certain sample types and dispersions, such as those containing surfactants or highly viscous carriers, might have a greater likelihood of bubble occurrence, in general, most bubble peaks occur inconsistently, which makes it easy to rule them out as part of the true particle size distribution.

ARTIFACT PEAKS
Also referred to as "ghost peaks", artifact peaks are well recognized among laser diffraction instrument operators, however, their technical cause is often poorly understood. Regardless, their occurrence can be identified and their associated cause can be discussed and should be investigated. Used here, the term "artifact peak" is meant as a general description to differentiate any outlier peak from the true particle size distribution that does not correspond to a tangible presence (such as a bubble, an unintended emulsion droplet, or agglomerated particles). Several common associated causes are discussed below.

THERMAL ARTIFACT PEAKS
Like bubble peaks, thermal artifact peaks are inconsistent in their occurrence. These form when volatile dispersing media do not reach thermal equilibrium within the instrument. Equilibration is usually accomplished by allowing longer recirculation times prior to taking a background measurement.
Figure 7 is an example of such an occurrence. Hexane, having a temperature of approximately 23ºC, was loaded into the instrument with an operating temperature of about 30ºC. The background measurement was taken before the hexane had reached equilibrium with the operating temperature of the instrument. The

sample was loaded into the instrument, and as the analysis progressed, the discrepancy between the background scattering signal and the active sample measurement scattering signal grew in correlation to the hexane's thermally adjusting to the operating environment. This signal discrepancy is caused by the slight change in refractive index of the hexane, because refractive index is temperature dependent. As this temperature difference between the initial measurement and final measurement increases in magnitude, so does the artifact peak at the coarse end of the distribution, which broadens over time until equilibrium is reached.
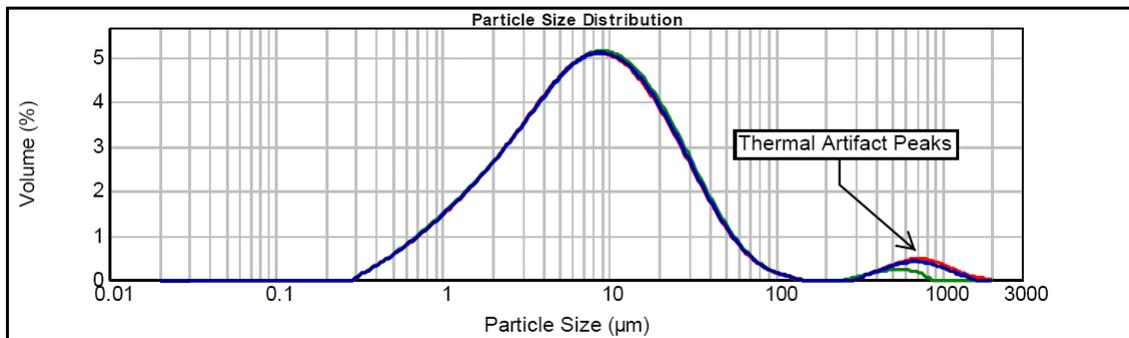

FIGURE 7. Thermal artifact peaks in volatile dispersing medium.

Thermal peaks can be avoided in most cases when proper precautions are taken to ensure that the temperature of a volatile carrier medium has reached thermal equilibrium within the recirculator of an instrument prior to taking a background measurement. When they do occur, they correlate to a peak at the coarse end of the distribution. The thermal difference results in "beam steering," where a larger signal reaches the inner or low angle detectors in some instruments. Depending on the size of the primary particulate, the thermal peak is often completely detached from the "true" particle size distribution. It should be verified by microscopy that primary particulate does not occur within the size range of the thermal peak in order to render the thermal peak suspect.

DRY DISPERSION ARTIFACT PEAKS
Similar to volatile liquid artifact peaks, dry dispersion artifact peaks occur at the coarse end of a dry dispersion distribution analysis due to the nonequilibrated state of the air current. An artificial peak can occur when the instrument's background measurement records a lower signal in the low angle detectors than in the signal captured during the sample measurement (unrelated to the signal produced by the actual sample particulate). Potentially exacerbated by lower air pressures, this occurrence can be associated with a rapidly fluctuating background signal, which, can be manifested as a peak at the coarse end of the distribution. Because of the nature of this dispersion technique and the operator's minimal ability to precisely control these fluctuations (as they are inherent to the technique), the problem can be difficult to manage at times. Occurrences may be minimized when steps are taken to stabilize and equilibrate the dispersion environment prior to analysis. Care must be taken to ensure that agglomerated particulate is not part of the problem and microscopic examination is necessary to verify the suspect peak is distinct from the primary particle peak. See Figures 8 and 9 for an example of a dry dispersion artifact peak and the corresponding photograph of the particulate.
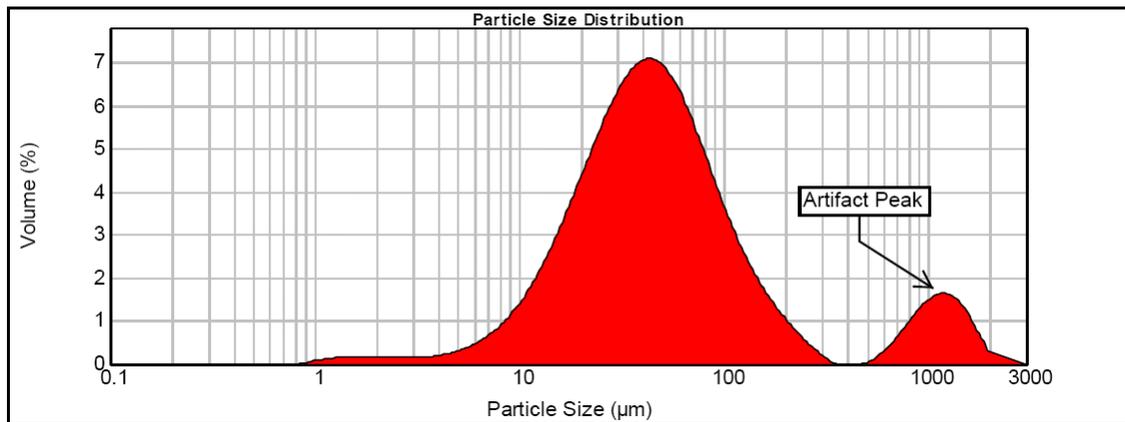
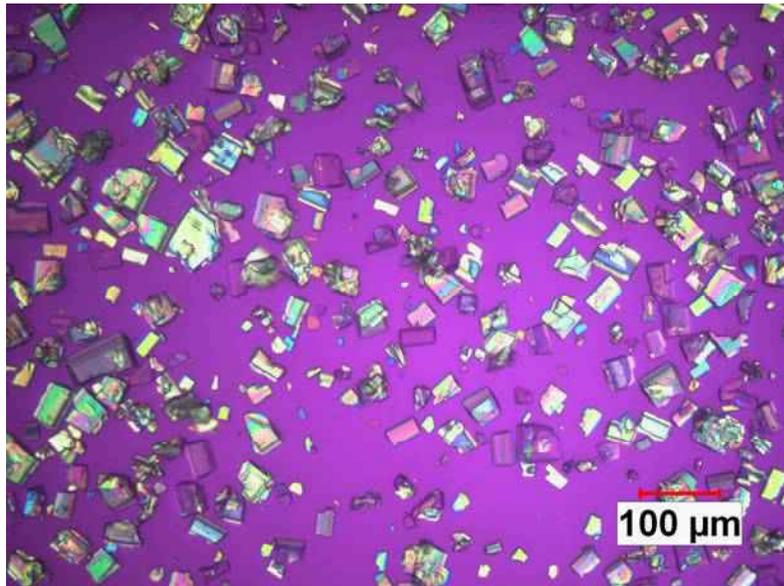**FIGURE 8**. Artifact peak in a dry dispersion analysis.

FIGURE 9. Photograph of particles in Figure 8. Note the absence of particles in the 500µm-2000µm range as compared to the distribution in Figure 8.

OPALESCENT/REFLECTIVE PARTICLE ARTIFACT PEAKS
In certain cases when a particle is particularly shiny, reflective, pearlescent, or otherwise opalescent, a coarse-end artifact peak may occur in a laser diffraction measurement. Figures 10 and 11 demonstrate this complication. The photo shows particulate around 10µm in size, while the particle size distribution shows an additional broad coarse end peak occurring at about 600µm, which is clearly not present as part of the particulate in the photo. The artifact peak is associated with the concentration of opalescent/reflective particulate within the recirculator of the instrument. Cutting back the concentration of the particulate is helpful in avoiding this occurrence, which can be sporadic over multiple measurements. Again, microscopic examination of the preparation is critical in the identification of this suspicious peak and in ruling it out as part of the particle distribution.
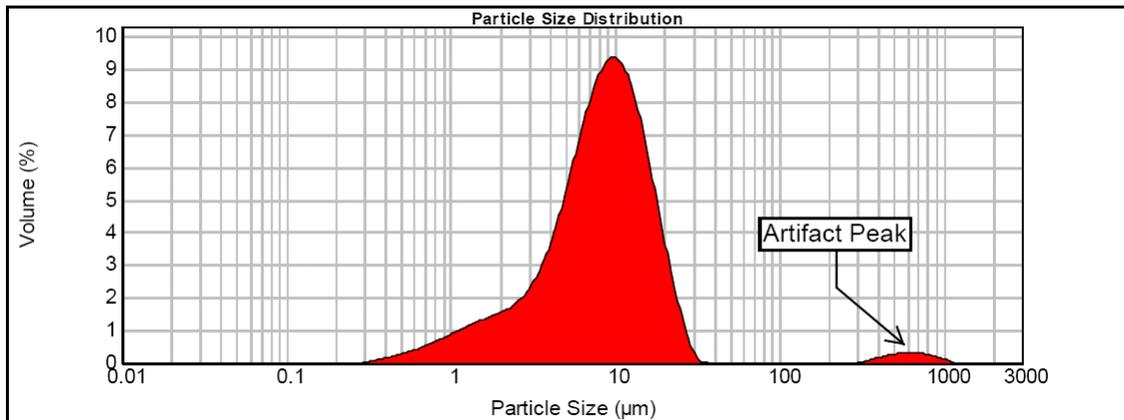
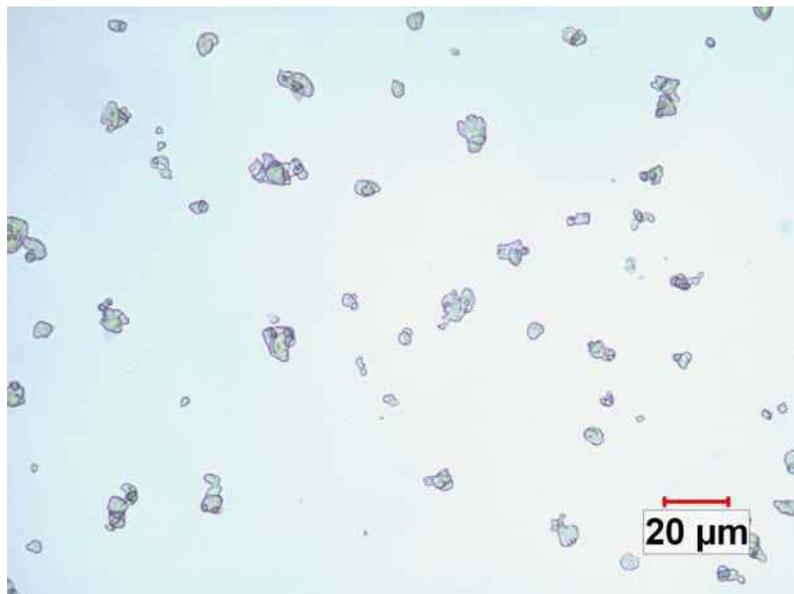**FIGURE 10**. Artifact peak caused by opalescent/reflective particulate.

FIGURE 11. Photograph of particles from Figure 10. Note the absence of particles in the 300µm-1000µm range as compared to the distribution in Figure 10.

OPTICAL MODEL ARTIFACT PEAKS

Of all the artifact peaks discussed within this article, optical model artifact peaks can be the most insidious because they can occur repeatably and consistently. While they follow a distinct pattern, they can be more difficult to scientifically rule out because of their particle size orientation and the limits of optical microscopy and other orthogonal (i.e. independent) methods. Luckily, unlike the other problematic peaks discussed, an optical model-related artifact peak does not invariably necessitate a complete reanalysis. Often a simple recalculation of the data is sufficient to remedy the problem.

Optical model artifact peaks are associated with the complex refractive index assigned to a particle for laser diffraction analysis using Mie theory. Mie theory, as opposed to Fraunhofer theory, assumes that the optical

properties of a particle are known, and so they must be entered into the instrument software as the particle's complex refractive index. International Organization for Standardization (ISO)13320:2009 defines the complex refractive index of a particle as consisting of both a real and an imaginary (absorption) component (4). The particle's refractive index (real component) can be directly measured using a Becke line test, for example. The imaginary or absorption component represents the reduction of intensity of a light beam that is not due to scattering. Particle properties, such as surface roughness, texture, and reflectivity; internal reflection or refraction; or color and opacity (i.e. actual absorption) can affect the beam intensity, thereby influencing the absorption value (4). To the author's knowledge, there is no commercially available method of directly measuring this absorption component as it specifically applies to laser diffraction; instead, it is inferred. Approximations can be made through volume concentration experiments in many instruments. Beekman et. al. provides an excellent discussion on the importance and impact of the imaginary and absorption component on laser diffraction particle size distribution data (5).

The laser diffraction document from ISO warns that an inappropriate choice of optical model may result in significant bias of the resulting particle size distribution (4). Figure 12 is an extreme example of an inappropriate choice of optical model. Generally, when the value selected differs greatly from the actual optical properties of the particle type, a detached or partially-detached peak may occur in the distribution, usually at the fine end (typically resulting in a sub-micron mode). While the particle's real refractive index value can contribute to this problem, the absorption value often poses a more significant influence.
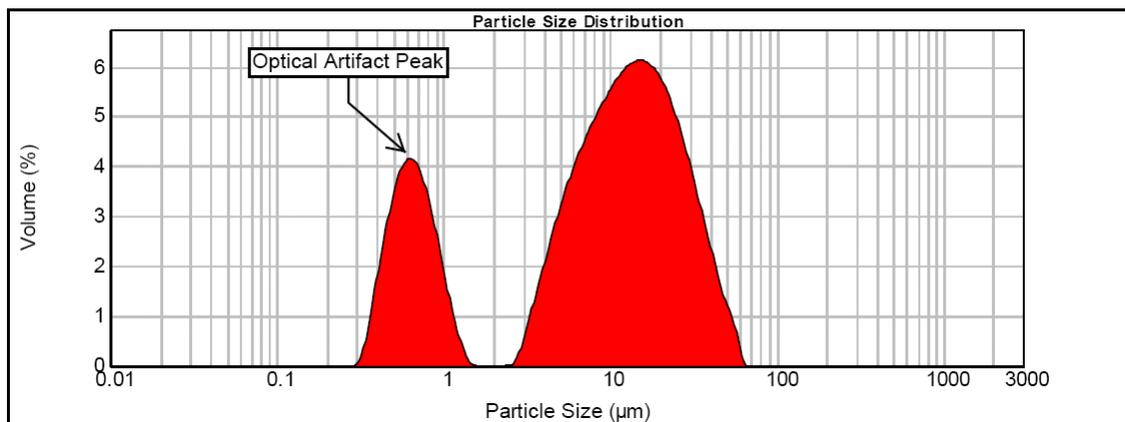

FIGURE 12. Optical artifact peak.

Simply changing the optical properties used for the particle drastically changes the resulting recalculated distribution, see Figure 13.
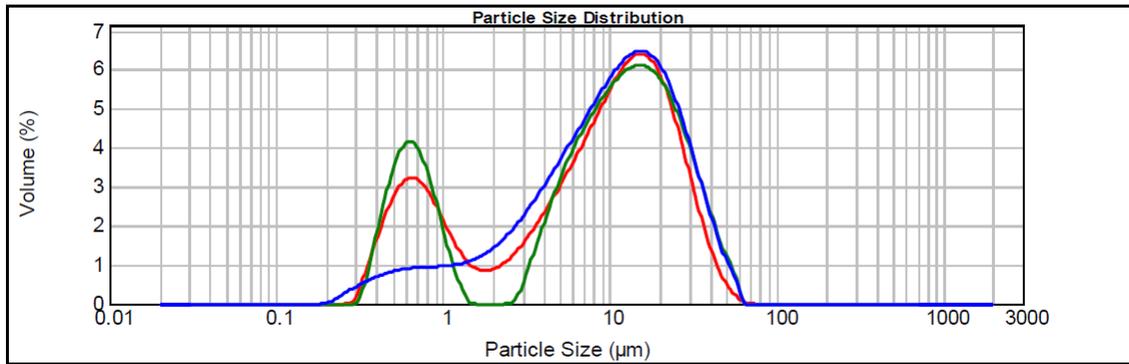
FIGURE 13. Influence of changing absorption values on a particle size distribution.

FIGURE 14. Photograph of particles from Figures 12 and 13. While submicron particulate is difficult to photograph due to the limits of optical microscopy, it can be observed as a haze or blinking on a live wet slide when viewed by microscopy. Nevertheless, it is unlikely that the distribution in Figure 12 is a realistic depiction of the particles in this sample.

As discussed previously, it can be generally assumed that most homogenous solid particle sample types should produce a continuous particle size distribution, so the occurrence of a distinct disconnected or partially-disconnected peak should be considered suspicious and thoroughly investigated. However, scientifically verifying the presence and relative volume of submicron particulate can be difficult as this pushes the lower limits of optical microscopy. As laser diffraction instruments provide data on a volume-basis, it is important to keep in mind that any fine particulate observed must be present in sufficient quantity to command a presence as a secondary peak alongside coarser particulate in the distribution. It is generally taught that submicron particulate of this concentration would exist in such quantities so as to produce a visible "haze" when observed under a darkfield microscope. While this haze is a good indication of the presence of submicron particulate, the human eye is not capable of making the final call as to the relative

volumetric proportions of the observed particulate. It is imperative that orthogonal testing techniques be utilized to either support or rule out the suspicious peak wherever possible. As a compliment to laser diffraction, such techniques include (but are not limited to): image analysis (microscopy), laser light obscuration, and electro-sensing zone, where possible and applicable.

While these techniques are valuable methods of distribution verification, their lower detection limits may decrease their ability to verify suspect peaks of entirely submicron orientation. When this is the case, indicators of fit (such as residual or weighted residual) can be useful in optimizing the optical model of best match to the measured data (6). Laser diffraction instrument software commonly includes an indicator of this nature and this can be very useful in assessing the quality and legitimacy of the data. It is important to note that the use of the residual can be used as one indicator when investigating an optical model, but is not a definitive form of verification.

CONCLUSIONS

When the particle size distribution of a material needs to be measured, steps should be taken to ensure that the measured data reflects the test sample rather than a bubble, artifact, or other source of data contamination. It is imperative that the physical nature of the particles is thoroughly investigated at the onset of method development and creation. Knowledge of the expected particle size is necessary to rule out any aberrations in the resulting data output. Optical microscopy and other orthogonal techniques are valuable tools for assessing and verifying the results of a measurement. Steps can be taken to prevent or minimize the occurrence of many common problems that manifest as erroneous peaks in the particle size distributions of laser light scattering instruments. Many of these problems are sporadic and otherwise inconsistent in occurrence, which can make them easier to identify with thorough testing. Problems that have the potential to cause inaccurate yet repeatable and reproducible results must be considered and managed with utmost diligence from the time of method inception. Thorough scientific scrutiny of suspicious results is necessary in order to either accept or reject measured data or a method. While laser diffraction instrumentation can be very useful for quality control, it is important that the results obtained by a given method are well understood and, ideally, are an accurate depiction of the intended particulate to be characterized.

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