

A decorative graphic consisting of several red dots of varying sizes arranged in a cluster, with a vertical dotted line extending upwards from the top dot.

How to Characterize Multimodal Samples using Particle Characterization Equipment

This application note will discuss the topic of size resolution in particle characterization and advantages and disadvantages of various techniques. Several demonstrations of complex, mixed samples in various instruments are provided. Readers will benefit from a deeper understanding of what to expect from a given product based on its technical underpinnings.

Introduction

A common customer request in the field of particle characterization is to characterize samples containing multiple size distributions mixed together in a single sample. This type of test provides an excellent demonstration of an instrument's resolving power and accuracy. However, the results expected from such a test depend upon a variety of factors such as the core technology, product design, sample preparation, and percentage difference in size of the underlying samples. While many experimenters may focus on testing two pure samples consecutively (eg, running 50 and 100 nm in two separate consecutive tests), it is much more difficult to test samples that are mixed together (eg, mixing 50 and 100 nm beads and resolving the peaks simultaneously).

This is a subtle, yet important distinction for customers. Most real world samples do not resemble the pure, monomodal polymer beads that are frequently used to test instrument performance. Rather, they are mixture of various sizes and components—and are not necessarily easy to separate into constituents. Therefore, it is most useful from an applications perspective if an instrument can resolve multiple component peaks from a single mixed sample.

Capabilities of Core Characterization Technology

In order to design meaningful experiments, it is important to discuss the various factors that can influence resolution with particle sizing equipment. The most important place to start is with the core technology itself—the maximum theoretical resolution of that technology is the best that could ever be expected in a product. Particle characterization uses a handful of core technologies to size particles, though there are many product iterations. The most popular techniques embodied in laboratory instruments are Laser Diffraction, Dynamic Light Scattering, the Coulter Principle, and various forms of imaging. Recently, some emerging technologies such as resonant mass measurement have appeared, but these are relatively limited in market penetration, and as such will not be discussed here.

Coulter Principle

The Coulter Principle relies on electrical impedance to size and count particles.¹⁻³ Briefly, electrodes are submerged into a conductive liquid bath on either side of a non-conductive barrier with only a small orifice which allows movement of liquid and electrical current across the barrier. This restriction of fluid and electrical flow creates a high density electrical field around the orifice— which can be thought of as a large quantity of electrical charge in a small volume. When particles are suspended into the liquid and pulled through such a high density electric field, they generate measurable electrical pulses. Each pulse corresponds to a single particle and the magnitude of the pulse is proportional to the volume of the particle which generated it.

The ultimate theoretical resolution of the Coulter Principle is extremely high—there are almost no theoretical limits. However, there are several practical limits such as electrical design, signal analysis, and signal to noise ratio. Still, even with these limitations, the best Coulter Principle instruments can achieve size resolution of a mixed solution better than 5%. As shown in Figure 1, the Multisizer 4e COULTER COUNTER can easily resolve 3 distinct particle populations centered around 0.500, 0.850, and 1.04 micron. Near 0.900 micron, the lower left hand edge of the 1.04 micron population and the upper right hand edge of the 0.850 micron population begin to overlap. Still, it is clear from the results that the system can distinguish easily between particles that are extremely close in size. This makes it an ideal choice for research applications and complex mixtures—where size resolution can be extremely important. Further, the simple, gentle natures of the technique make it ideal for analysis of cells and biological materials.

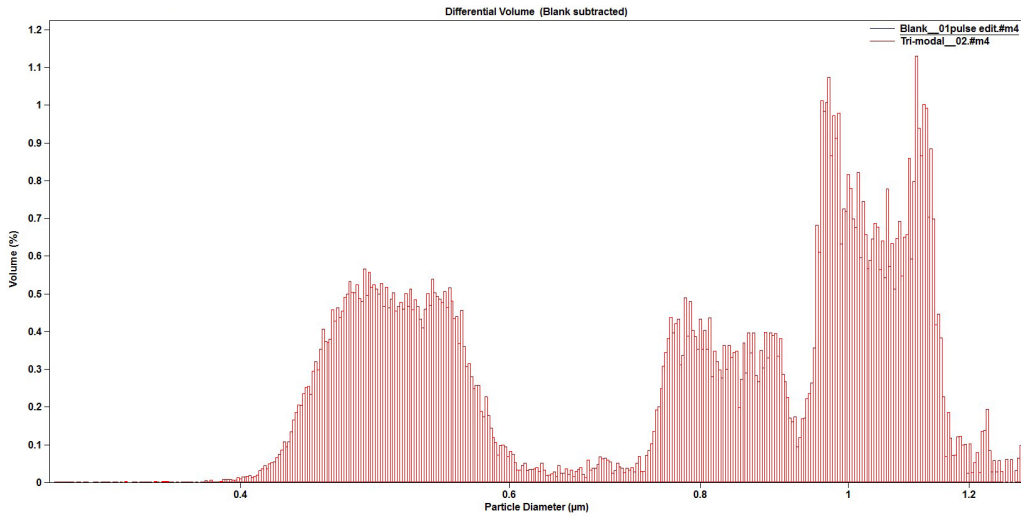


Figure 1. Size Histogram of a mixed sample measured on the Multisizer 4e.

Laser Diffraction

Laser diffraction relies on the physical principles of static light scattering to analyze particle size distributions. Several extensive publications exist on the theoretical underpinnings of laser diffraction.⁴⁻⁵ In a brief summary, laser diffraction instruments measure and analyze the intensity and angles at which incident light is scattered by populations of particles. There are several mathematical models which can be used to model this behavior and arrive at a size distribution of the particle populations. The ultimate theoretical resolution of the technique itself is a function of the laser wavelength, the spacing of the detectors, and the optical design of the system (which consists of several technical details such as detector area and signal-to-noise ratio). For example, an X-RAY diffraction system uses ultrasmall wavelengths of light to achieve subnanometer resolution on a continuous detection system. Because there are several practical limitations to using X-RAYS in an industrial quality control environment, particle sizing equipment uses lasers with visible and near-visible wavelengths of light.

Thus, because the wavelengths of the equipment are similar in all instances, the fundamental mathematical relationships are similar, and all systems feature high quality components, the number of detectors and angular spacing of those detectors becomes very important to system resolution. That is because more detectors per scattering angle provides more data points into the mathematical relationships to achieve an accurate fit. For a mixture, laser diffraction systems should be able to provide peak-to-peak resolution of 25%-50%, depending on the size range and number of detectors at particular angles. Figure 2 shows the results of a mixed sample of 30 micron and 40 micron beads, or about 25% resolution peak to peak. The results are clearly lower resolution than the Multisizer results above—which is why the peaks appear much smoother. However, the laser diffraction instrument can clearly delineate the two peaks. While its resolution is lower than that of the Coulter Principle, laser diffraction has several attractive qualities such as simple operation and broad dynamic range that make it an ideal choice for industrial applications.

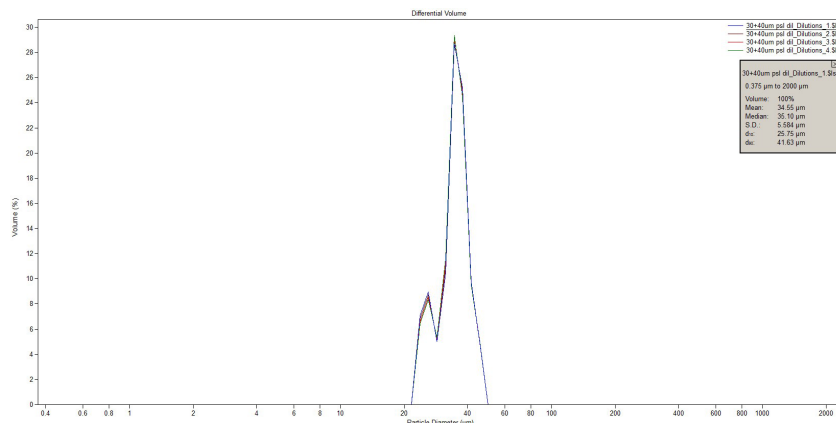


Figure 2. Size Histogram of a mixed sample measured on the Multisizer 4e.

Dynamic Light Scattering

Dynamic Light Scattering relies on diffusion to analyze particle size. Briefly, in a dynamic light scattering experiment, particles suspended in a liquid are placed in a laser path with a single detector at some angle of interest (typically 90 or 165 degrees). As the particles diffuse around the liquid, floating in random directions, they occasionally pass through the laser focal volume. While traversing the focal volume, they scatter light in many angles, including the angle at which the detector is placed. The result is intensity fluctuations of scattered light at the detector. The rate at which these fluctuations occur is proportional to the particle size (larger particles diffuse slower and have less frequent fluctuations). The mathematical formulation to derive particle size from random intensity fluctuations is quite complex, and has been well described elsewhere.⁶⁻⁷

However, from the description of the experimental set up, several key insights related to ultimate resolution are available. First, there is only one indirect detection system for the entire population of particles—thus, it can be difficult to ascertain multimodal particle size distributions distinctly from broad Gaussian distributions. Second, resolution can be complicated by the huge difference in scattering intensity between large and small particles. Scattering intensity is proportional to particle diameter to the 6th power ($\times 10^6$) which means that a single large particle scatters the same amount of light as millions of small particles. Finally, it should be clear that the mathematical formulation used to determine the particle size has a dramatic effect on the ultimate resolution. Typically, dynamic light scattering instruments claim a peak to peak resolution on the order of 300%-600%. Figure 3 shows that the DelsaMax System from Beckman Coulter can easily resolve a mixture of 80.3, 199.1, and 506.2 nm nominal diameter beads (147% peak-to-peak resolution for 80.3-199.1 and 154% peak to peak resolution for 199.1-506.2). This result shows the superior resolving power of this system, and is difficult to duplicate, if not impossible, with other dynamic light scattering systems.

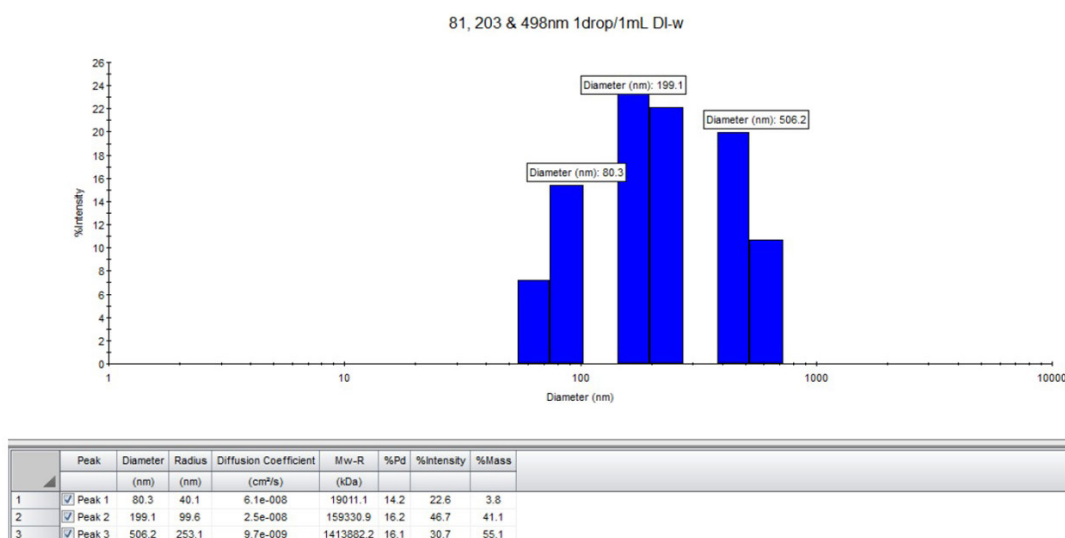


Figure 3. Size Histogram of a mixed sample measured on the DelsaMax.

Imaging

Imaging is another technique with very high theoretical resolution. With very high resolution cameras and superior optical design, imaging systems should be able to achieve peak-to-peak resolution of just a few percent. However, image analysis software, and a host of practical problems can limit the ultimate resolution. These include lens aberrations, fouling/dust, light source power fluctuations, analysis algorithm ambiguity, and low relative sample quantity. While the first three factors are relatively straightforward to understand, analysis algorithm ambiguity amounts to difficulty discerning the true boundary of a particle through pixel-by-pixel analysis. Usually, these boundaries are established by noting color changes (eg light to dark) in the pixels. However, there can sometimes exist ambiguity especially if the particle is out of focus. Secondly, imaging typically has much lower statistical confidence than the other techniques described above. In a typical image analysis, a few thousand particles is considered a large number. However, with the Coulter Principle, Laser Diffraction, and Dynamic Light Scattering, millions of particles can be analyzed providing statistical confidence. Still, even with these caveats, imaging can provide excellent resolution if instruments are well designed.

Demonstrated Quality of Beckman Coulter Instrument Design

Perhaps one of the most satisfying results of analyzing particles with multiple instruments employing different technologies is when all of the results are in agreement. This indicates an excellent understanding of the true sample size distribution and also shows the instruments are well suited to the task of characterizing complex, unknown samples. To demonstrate the quality and accuracy of Beckman Coulter instrument design, we tested a complex submicron mixture on three completely different platform instruments: our Laser Diffraction (LS 13320), Coulter Principle (MS4e), and Dynamic Light Scattering (DelsaMax) products. Figure 4 shows that all the results are in excellent agreement. The nominal bead diameters were 81 nm, 203 nm, and 498 nm. The Multisizer 4e clearly shows the 203 nm and 498 nm peak, but the 81 nm bead is below its lower detection limit of 0.200 micron. It is important to note that this lower limit is the lowest for any commercially available COULTER COUNTER.[®] Thus, the 81 nm particle is not resolvable as it falls below the noise floor. Still, the fact that all three instruments can resolve these complex mixtures shows that Beckman Coulter Characterization equipment has superior design and performance characteristics and should provide the utmost confidence in our results.

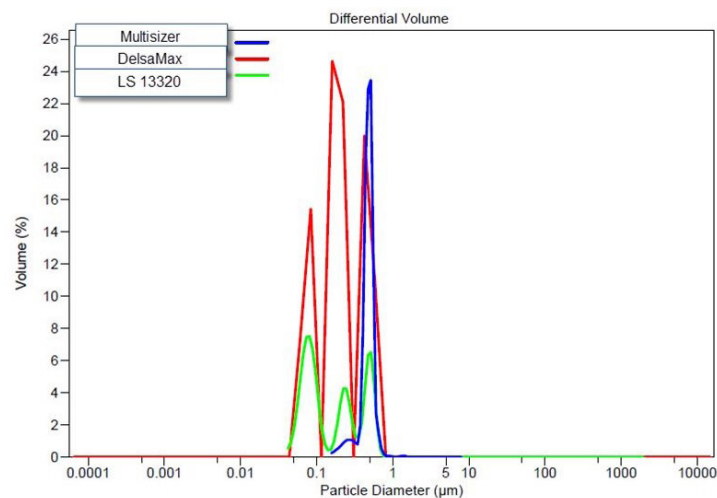


Figure 4. Size Histogram of a mixed submicron sample on all the DelsaMax, LS 13320, and Multisizer 4e.

References

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