HORIBA

Explore the future

Method Expert: Guided, Automated Method Development for the LA-950/960

Introduction

Developing an appropriate method for measuring particle size distribution in liquids using laser diffraction requires a structured approach. Samples analyzed as liquid dispersions include suspensions, emulsions, and solids dispersed in liquid. These three general categories of samples have a few unique considerations, but the majority of the content in this document will be applicable to all.

Suspensions and emulsions can sometimes be easily measured using the continuous phase of the original sample as the diluent in the analyzer. Proper dispersion of powders into a liquid may require the additional efforts of wetting and stabilization.

In all cases the basic goals remain the same; decide what the purpose of the measurement is, place a representative sample into the analyzer, disperse the sample in a liquid that does not dissolve or alter the particles, choose appropriate system settings for the measurement, and test for repeatability and reproducibility.

Sampling

It is rare that the entire sample brought into the lab is measured in the instrument. More typically a sub-sample of the total is analyzed, creating the need to consider the sampling technique.

Several references (1,2) can provide both background information and practical suggestions on proper sampling techniques. Too many scientists simply shake a suspension or tumble a powder sample and then remove a portion for analysis. Ignoring the sampling component of the method is inappropriate for several reasons:

- Accepted standards stress the importance of sampling. ISO 13320 (3) and USP <429> (4) both advise that a representative sample be prepared using a sample splitting technique. (5)
- One of the goals of proper method development is to minimize the total error. If sampling is ignored the developer doesn't know which portion of the total error comes from the sampling.

Care must be taken with sampling when dispersing a powder into a liquid. Many methods call for pre-dispersing the powder in a beaker and then pipetting the sample into the analyzer. When following this approach it is better to mix a concentrated paste in the beaker in order to minimize sampling bias during the transfer to the analyzer. If this is not practical for whichever reason, then the beaker pre-dispersion should be continuously stirred and sample should be extracted halfway between the center of the beaker and the wall and also halfway between the liquid surface and the bottom of the beaker.

Choosing the Solvent (diluent)

Suspensions and emulsions can sometimes be easily measured using the continuous phase of the original sample as the diluent in the analyzer. When powders are dispersed in liquid the solvent must meet the following criteria:

- Negligible reactivity with powder, defined as:
  - Does not swell or shrink particles by more than 5% in diameter (6)
  - Solubility must be less than 5g powder in 1 kg liquid (6)
- Have a refractive index (RI) different than the sample
- Be free from bubbles & particles
- Have suitable viscosity to enable recirculation (not required for some LA-950/960 sample handler accessories)
- Be chemically compatible with materials in the analyzer

Dispersing powders in liquid can often present challenges. The ISO 14887 standard (6) provides useful insight into this realm. Among the suggestions in ISO 14877 is to prepare the sample on a slide and look at it under a microscope. Determine if you are looking at individual particles or clumps. See if exposing the sample to an external ultrasonic probe eliminates the clumps.

Surfactants are often required to wet the powder for proper dispersion. ISO 14887

provides a comprehensive listing of commercially available dispersing agents. The HORIBA applications lab makes frequent use of many surfactants including Micro 90 solution (also good for cleaning the instrument), Triton X-100, Igepal CA630, Tween 80 and lecithin.

Once a powder is dispersed, it sometimes helps to add a stabilizer (or admixture) to the sample, such as sodium hexametaphosphate. The stabilizer alters the charge on the surface of the particles, preventing re-agglomeration.

Stability Testing

After the dispersing liquid or mixture has been chosen, test the system for stability by collecting multiple measurements as a function of time. Measuring the recirculating sample should generate extremely reproducible results.

Measure the sample at least three times over a time frame of several minutes. Check the coefficient of variation (COV, 100*Std Dev/Average) for the multiple runs and investigate those data which have COV > 1% at the d50.

A particle size distribution which steadily shifts finer alongside an increase in light transmission may indicate dissolution. An increase in particle size may indicate agglomeration or swelling. An increase in transmission alongside the disappearance of the coarsest particles may indicate settling. Random variations are more difficult to interpret but could arise from thermal fluctuations or poor mixing.

Determine the Refractive Index

It is important to determine an appropriate refractive index (RI) value for the sample and diluent during method development. It is better to determine the RI early in the method development process in order to understand the general shape of the distribution before conducting other tests.

The RI of the diluent is typically found in the instrument software library, or can easily be measured or found in published tables. Methods to obtain the real RI value for the sample include literature and internet searches, use of an Abbe refractometer, RI matching liquid and Becke line testing, and sending samples to third party laboratories.

Selecting the imaginary component of the RI can be more challenging. The simplest option is choosing zero for transparent spherical particles like glass beads or emulsions. Non-transparent particles can have imaginary values ranging from 0.01 – 10. In practice the choices can typically be distilled down to the values of 0, 0.1, 1.0 and 10. In the past many customers would measure the sample using the known real RI component, re-calculate the results using different values for the imaginary component, and the result that matched expectations was chosen. This process could be facilitated by also checking the residual *R-parameter* value calculated in the LA-950/960 (an error calculation that compares measured vs. theoretical light scattering) software. A lower R value indicates a better optical model selection, and therefore a lower error when converting the scattered light data to a particle size distribution. (7)

The process of re-calculating results using different imaginary RI values and searching for the lowest R value is now automated using the LA-950/960 Method Expert software package. The user selects the real RI value, the imaginary values to be investigated, and the calculations are automatically performed. Once the calculations are completed the results are then presented in a variety of formats (see Figure 1), allowing the user to make an informed decision based on both reported particle size distribution (PSD) and minimum R value.
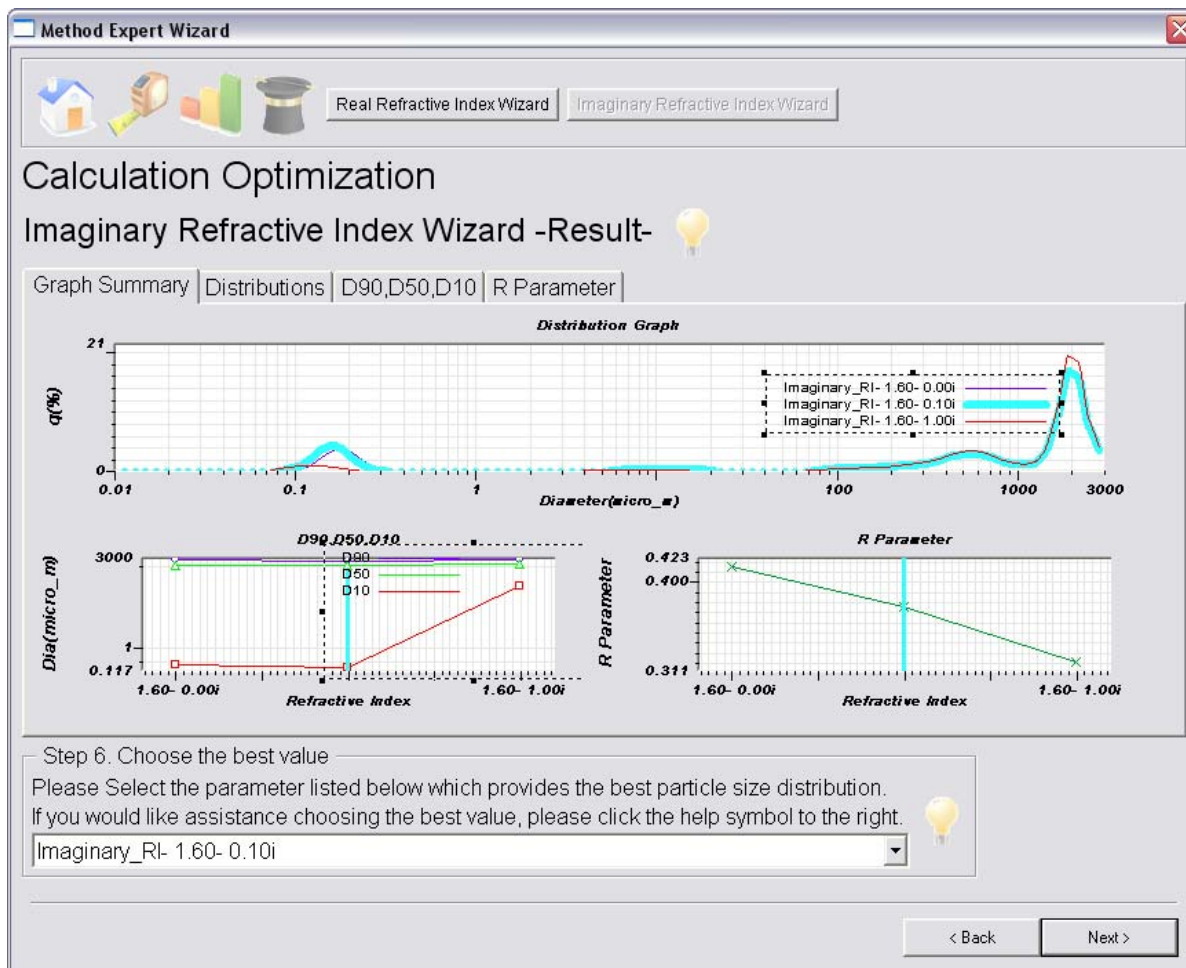
Figure 1: Result screen for the Imaginary Refractive Index Wizard

The RI Calculation Wizard can also be used to select the real RI value for sample by fixing the imaginary RI component and varying the real RI component. An example for this approach might be an oil in water emulsion where the real RI value of the oil phase is not known. Since the imaginary RI value should be zero the user can set this value, and then calculate multiple results using a range of real RI component values.

Circulation Pump Speed

After the refractive index is selected the next step should be to determine if pump speed affects the measured particle size distribution. Using a pump speed that is too slow may not adequately suspend large and/or dense particles. Using a pump speed that is too high may either introduce air (bubbles) into the system or break fragile particles. The range of pump speeds in the LA-950/960 goes from 900 to 4900 RPM which corresponds to 15 distinct speed values in the software. It is best to test the sample across a broad range of speeds such as 1, 4, 7, 10, and 13. It is rare that only a single pump speed will work for a given sample. If speed 4 works well, then 3 and 5 would probably also be appropriate.

The Method Expert software automates these tests either going from low pump speed to high, or from high to low. Starting at a low pump speed and then increasing is recommended for samples where there is concern the particles might break at higher speeds. Starting at a high pump speed is recommended for large/dense particles so the user can observe when settling begins to

take place as seen by a decrease in the reported PSD. The set up of these tests is shown in Figure 2 and an example of reported results in Figure 3. In this example the user would choose setting 13 based on the reported PSD. Once this selection is made the value is stored as part of a method file that will be used to automatically measure this sample in the future using all of the chosen instrument settings. The results from each step of the method development can be stored and/or printed in order to document the completed test and chosen parameter.
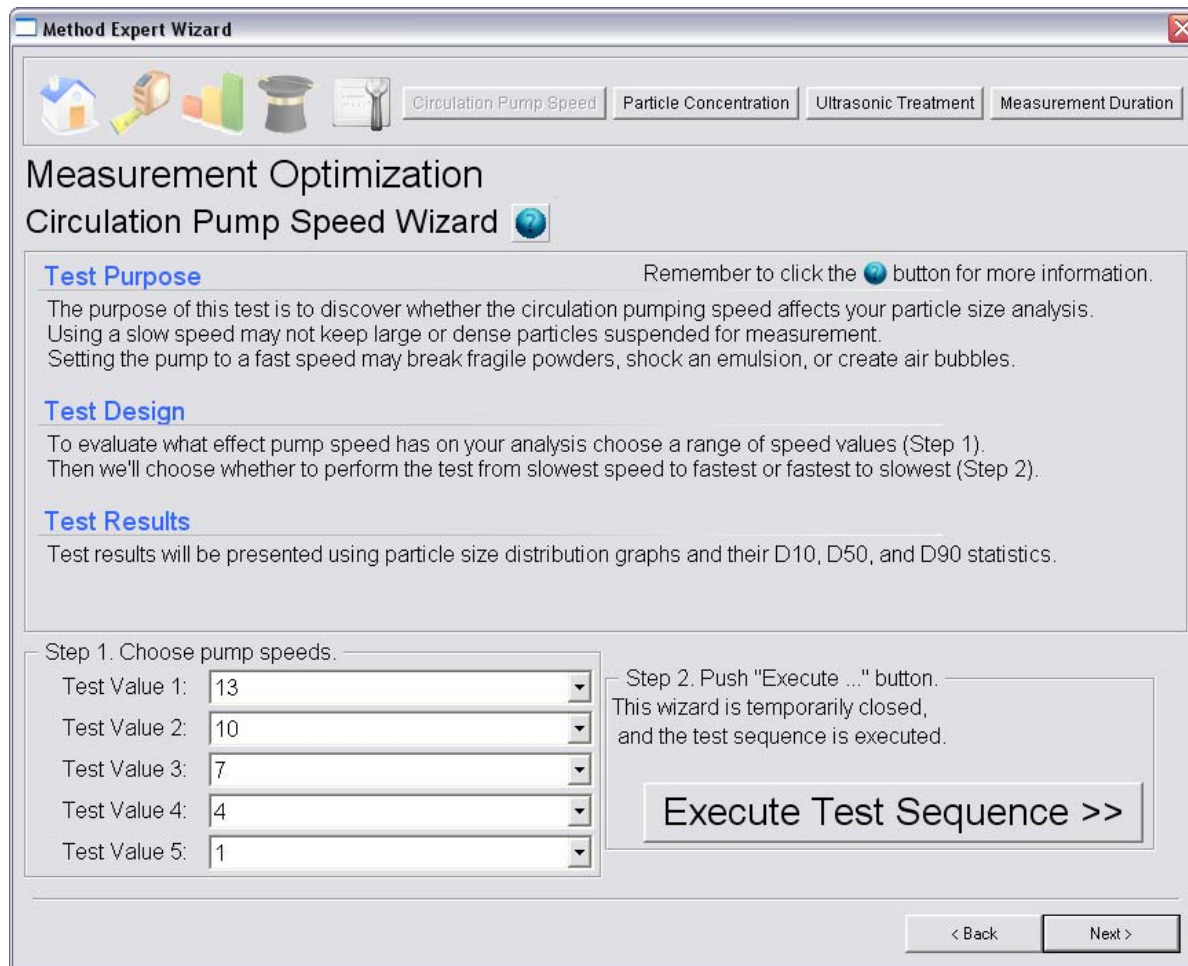
Figure 2: Setup screen for the Circulation Pump Speed Wizard
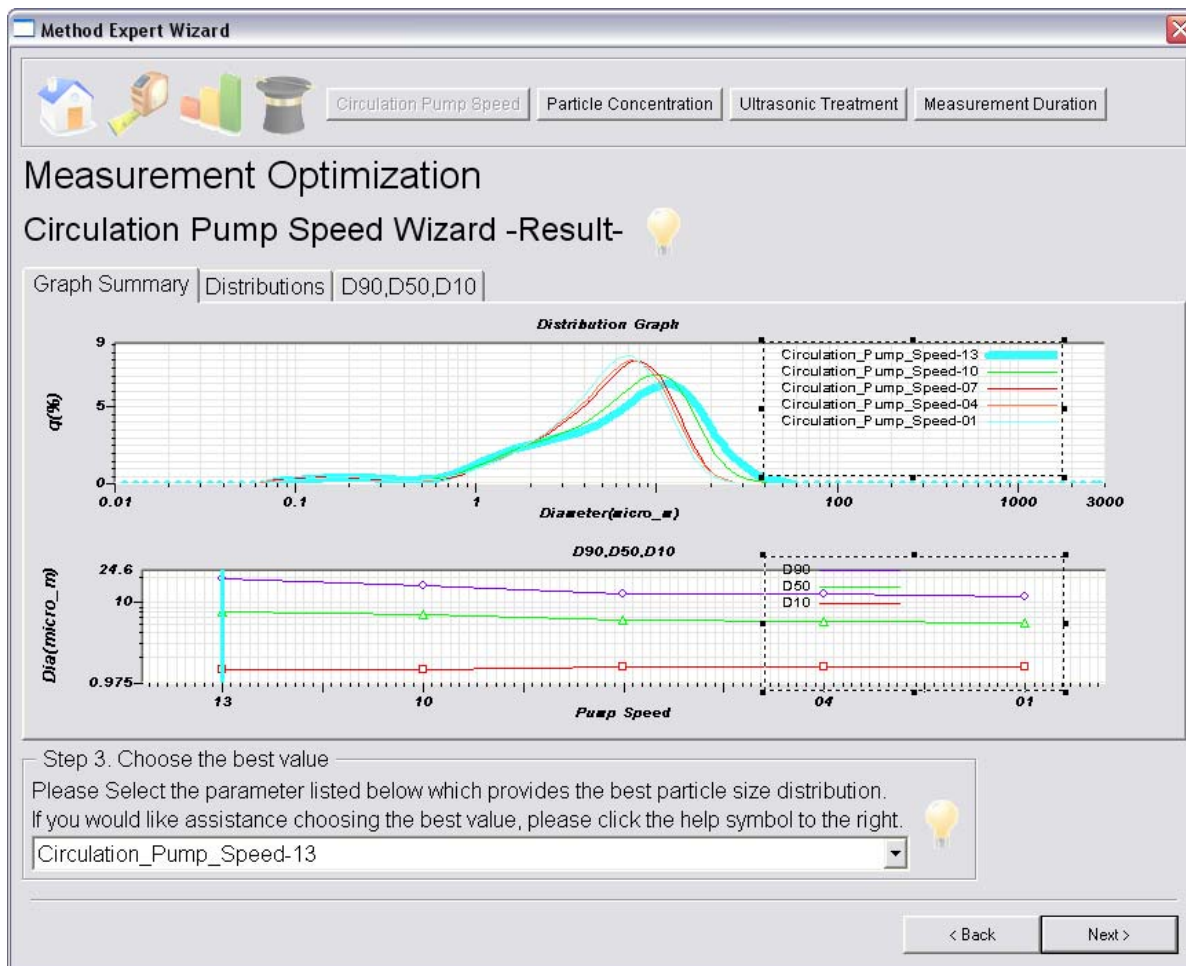
Figure 3: Result screen for the Circulation Pump Speed Wizard

Particle Concentration

The operator can typically control the sample concentration during a particle size analysis measurement when using laser diffraction. When measuring suspensions the operator adds sample to the recirculating diluent until a desired concentration is achieved. The LA-950/960 reports sample concentrations as percent transmission (%T), which is the average percent of the initial laser intensity detected during the measurement. The initial laser intensity is detected and stored after alignment and the background reading. This is the laser intensity with no particles present, or 100 %T. This value decreases as particles are added to the system. A 90 %T indicates that 10 % of the initial intensity has been blocked (diffracted, refracted, absorbed, or reflected) by the presence of particles.

Results vary with concentration on all instruments for all samples, and the variation is sample dependent. Factors influencing the variation include particle size, width, and optical design of the instrument. During method development it is important to investigate the effect of concentration on results generated. According to the ISO standard for laser diffraction[1]: "The particle concentration in the measurement zone should be high enough to produce an adequate signal (or in other words to reach an acceptable signal-to-noise ratio with respect to precision), yet low enough to ensure multiple scattering to be insignificant to the particle size result". Enough sample must be added so that the signal-to-noise ratio is high enough that the results are reproducible and accurate. But this must be

balanced with the effect of multiple scattering causing a decrease in reported size if the concentration is too high (%T too low). (8)

The selection of optimum concentration is automated within the Method Expert software. The user can select up to five concentration values within chosen upper and lower limits as shown in Figure 4. The test can be performed based on the concentration values measured by either the red or blue light sources used in the LA-950/960. Almost all samples should be studied based on the red light source, but certain sub-micron samples may be better optimized based on the blue light source.
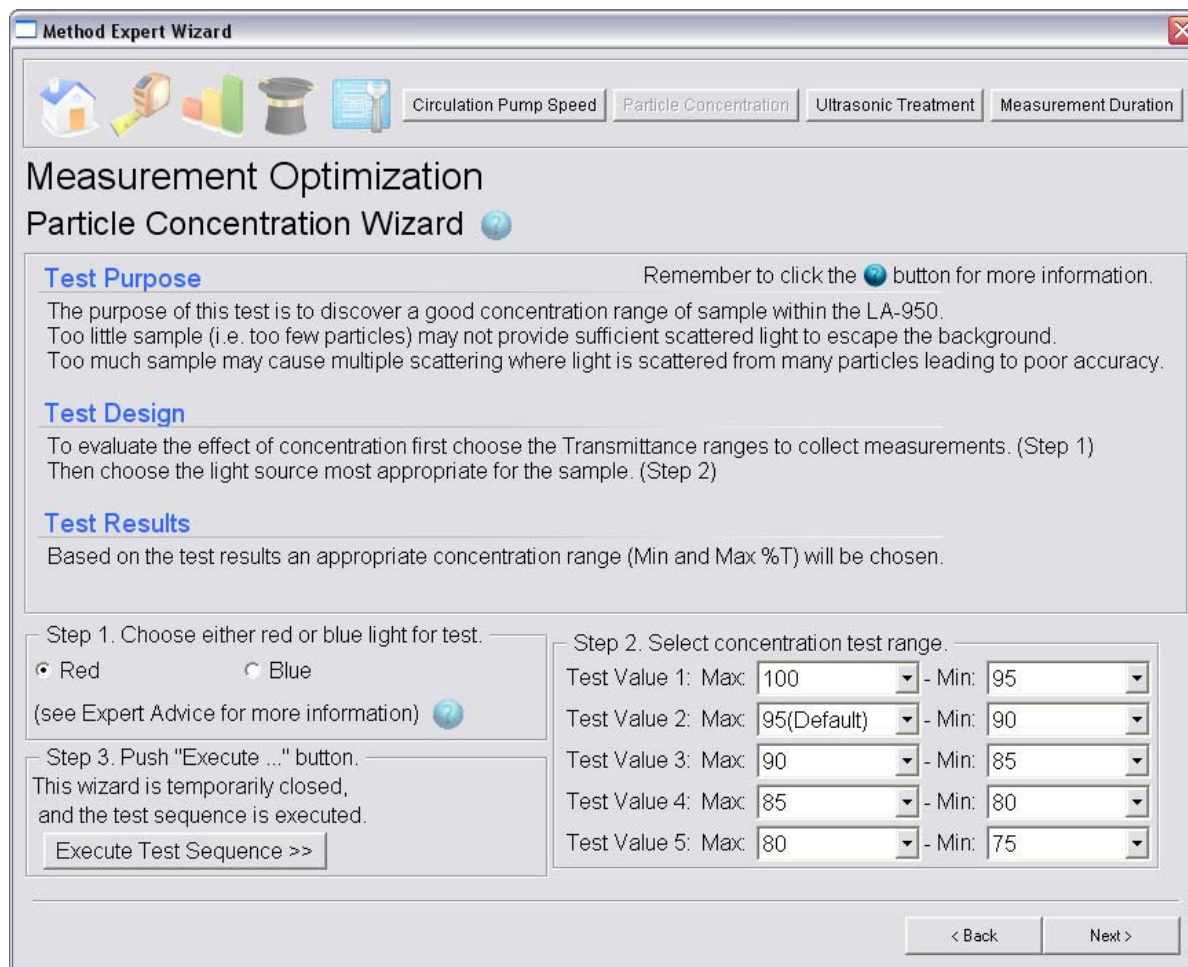
Figure 4: Setup screen for the Particle Concentration Wizard

This test is best performed starting at a low concentration (~95 %T) and then adding sample to raise the concentration. Once the tests have been completed the results are displayed as shown in Figure 5.
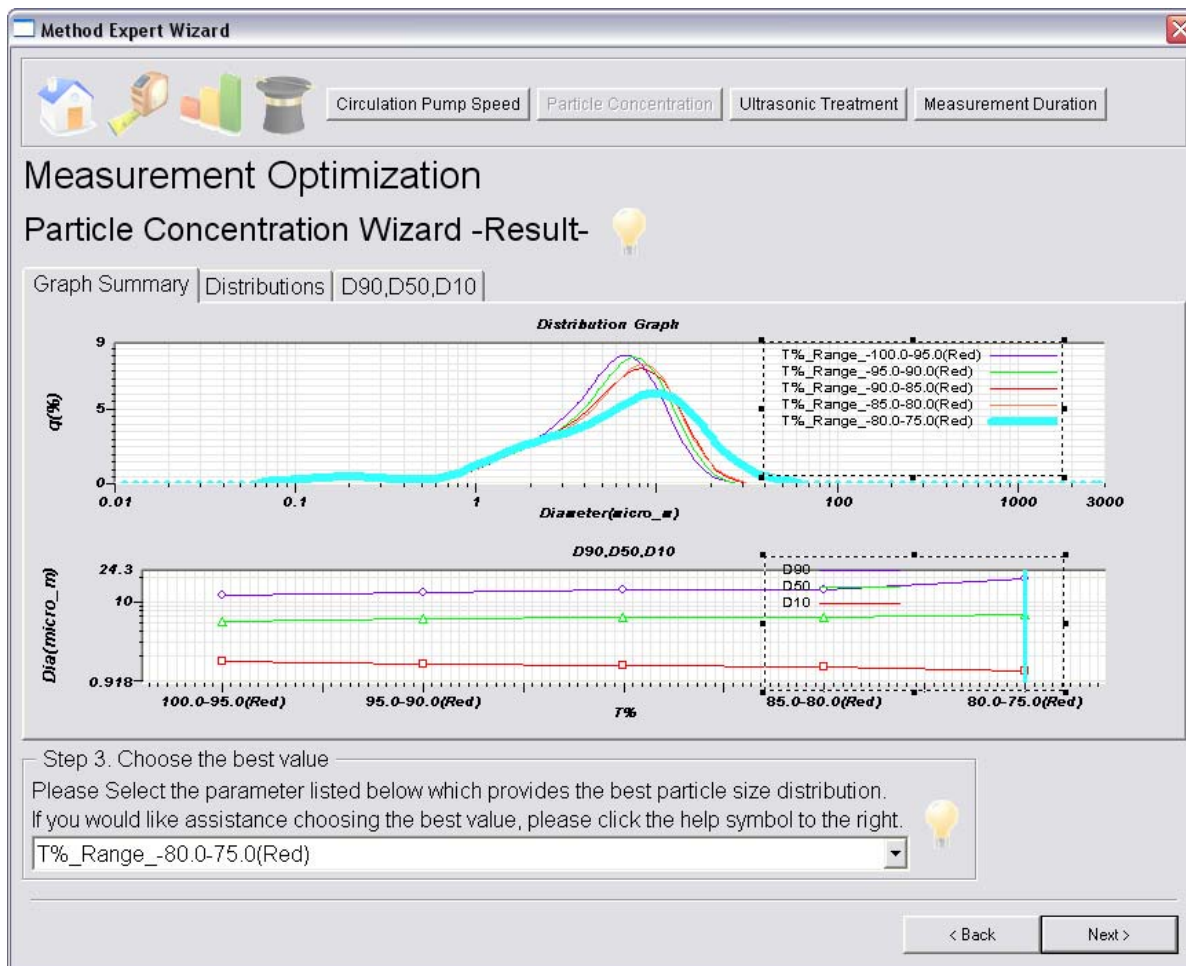
Figure 5: Result screen for the Particle Concentration Wizard

An example of the effect of multiple scattering is shown in Figure 6 where %T is plotted vs. particle size for the d10, d50, and d90. Multiple scattering begins to artificially reduce the reported PSD when the %T falls below ~75%. Notice that the d10 is affected more than the d50 or d90. This is because multiple scattering begins to affect results for smaller particles before larger ones.
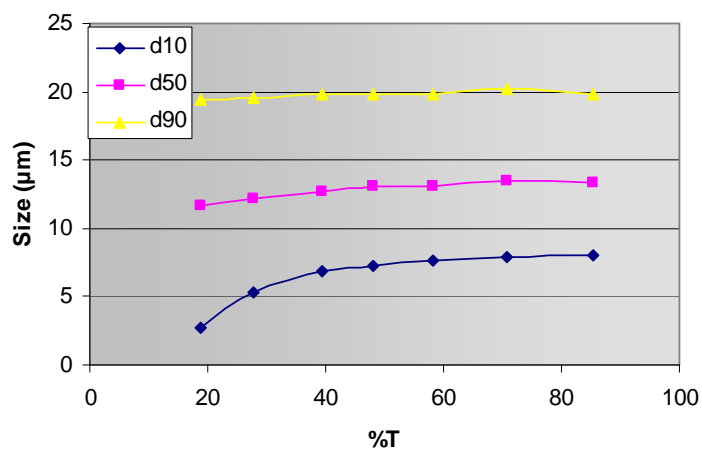

Figure 6: %T vs. reported particle size

Using Ultrasound

Ultrasound is frequently used as an energy source for the dispersion of agglomerated particles to the single (or primary) particle state. The LA-950/960 has a built in ultrasonic probe to aid dispersion. The amount of energy added to the system can have a significant effect on the reported PSD. In this case the instrument setting (amount of ultrasound) is altering the state of the particles being measured. The goal in method development is typically to disperse the sample to the single particle state without fracturing individual particles. The choice of optimum energy level can often be made by examining the two tails of the distribution. Larger particles disappearing with the addition of ultrasonic energy typically indicates the desired dispersion of agglomerates. Smaller particles appearing with the addition of energy typically indicates the unwanted breakage of individual particles.

Figure 7 shows the effect of varying the amount of time ultrasound was applied to a sample of microcrystalline cellulose (MCC). Result with no ultrasound (red) and 5 sec ultrasound (blue) include agglomerates. After 10 sec of ultrasound is applied (black) the agglomerates have been dispersed to the single particle state. Additional ultrasound exposures of 15 and 20 seconds generate similar, well dispersed results. After 40 and 90 seconds of ultrasound (purple and yellow) new smaller particles appear at the fine tail of this distribution. This fine tail is interpreted as indicating the unwanted breakage of individual particles. For this sample ultrasound application for 10-20 seconds appears to be ideal. A selection of 15 seconds would be practical in this case, and a further study of reproducibility under this condition may be the next step.
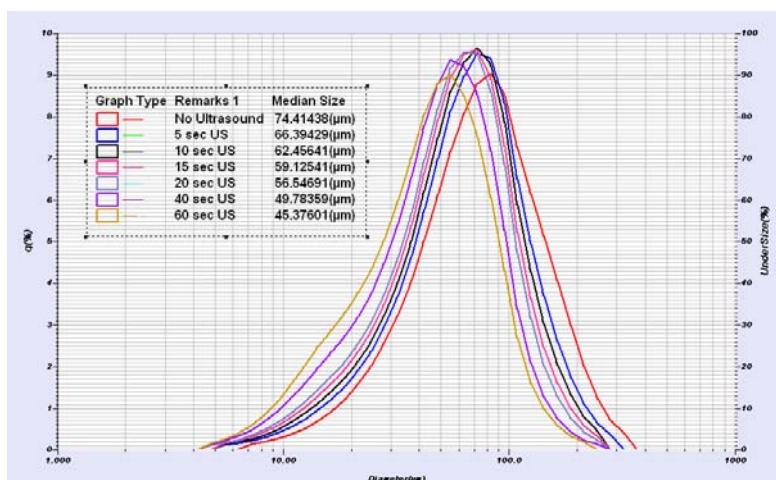

Figure 7: Reported PSD as a function of applied ultrasonic energy

The amount of energy added to the sample can be controlled two ways; varying the power setting of the probe for fixed time, or fixing the power setting and varying the amount of time the probe is turned on. The Method Expert software allows the user to investigate optimum settings using both approaches. The example described above and shown in Figure 7 fixed the ultrasonic probe at the maximum setting (7) and then added energy in 5 second increments (not all data is shown). This is a common experimental sequence. The screen for designing the ultrasound study is shown in Figure 8. In this example the power is set to maximum (7), ultrasound is applied for 10 seconds, a measurement is made, and this sequence is repeated five times so the sample is exposed to a total of 50 seconds of ultrasound by completion of the study.
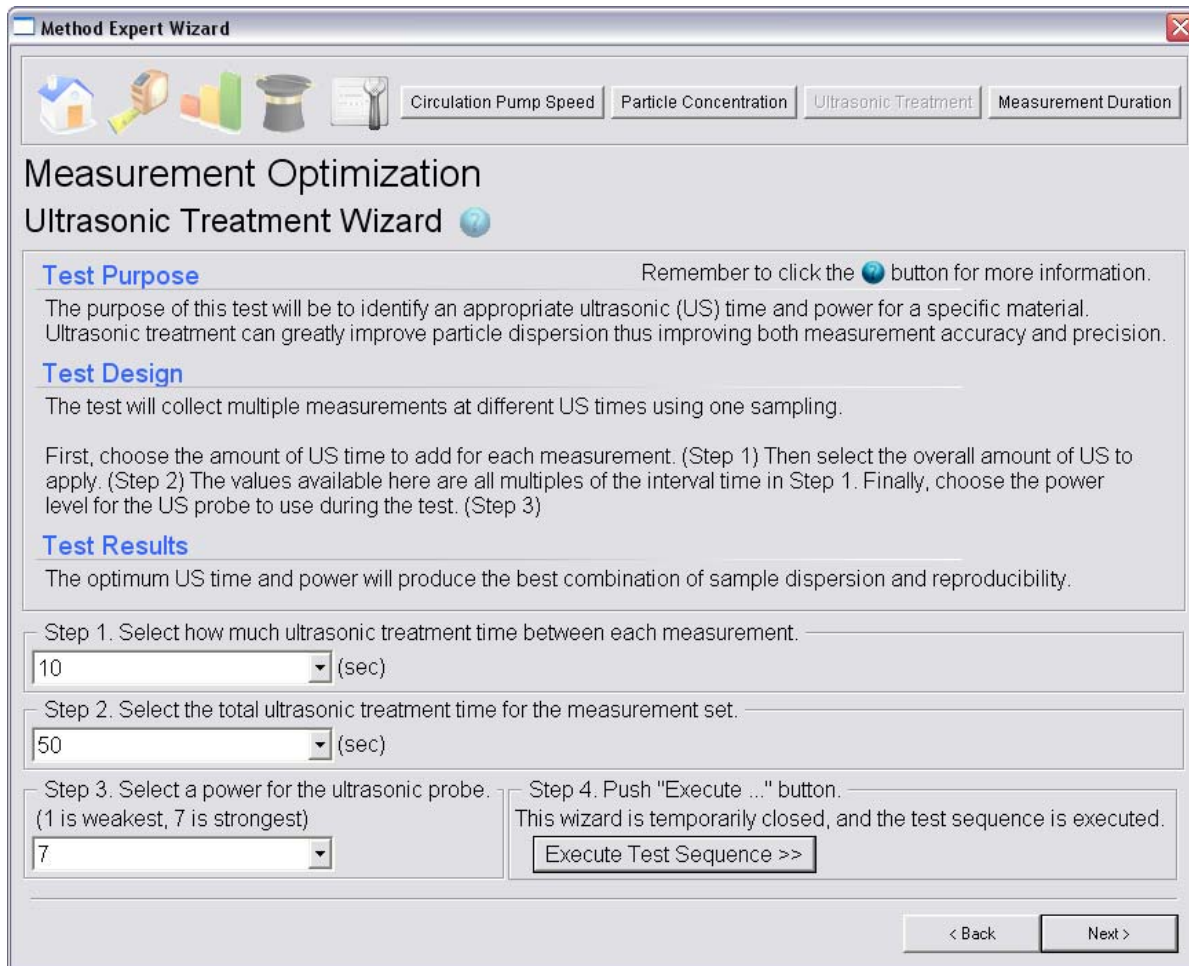
Figure 8: Setup screen for the Ultrasonic Treatment Wizard

After the test sequence is executed the software displays the results through a variety of formats and the user chooses the optimum amount of ultrasound for the sample as seen in Figure 9.
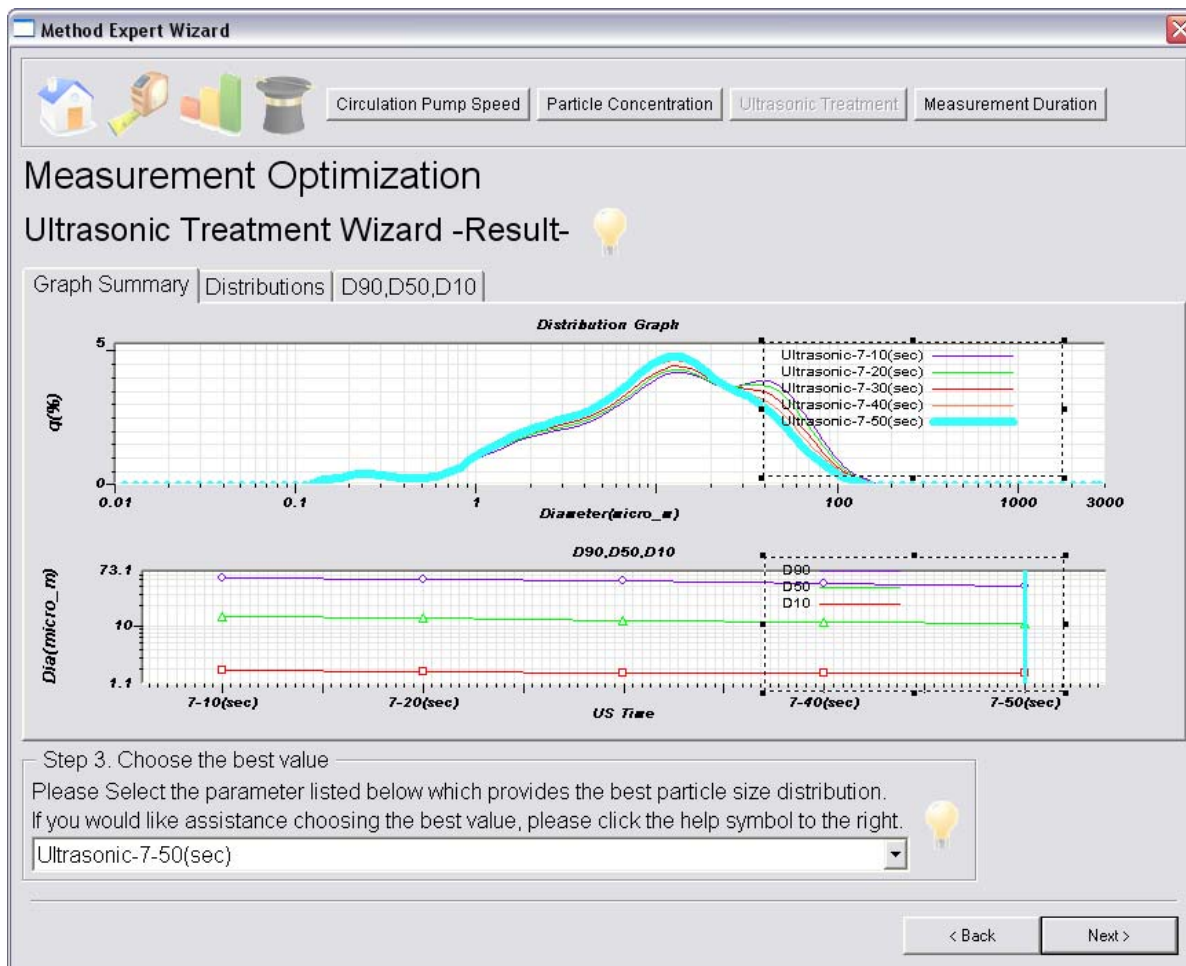
Figure 9: Result screen for the Ultrasonic Treatment Wizard

Measurement Duration

The last step in method development is choosing the length of time to collect the measurement. Since laser diffraction is an ensemble technique many thousands of particles are analyzed during a measurement. Increasing the measurement duration increases the number of particles inspected. The default measurement duration setting for the LA-950/960 is 5000 (scans of the detector) which only takes two seconds. This is sufficient for most samples, but should be increased for broad distributions or large particles. Samples with a broad distribution such as soils should be analyzed for a longer time in order to inspect a sufficient quantity of particles in all size ranges. There are far fewer large particles per unit volume than small particles at a given concentration, so samples with large particles (>100 µm) should also be measured for a longer time. The goal of the measurement duration study is to determine the time required to generate reproducible results that include the entire particle size distribution. Multiple consecutive measurements which show an inconsistent distribution feature typically indicate a measurement duration which is too short. The user should select at least three duration values as seen in Figure 10 and choose the shortest measurement that includes the entire distribution (Figure 11).
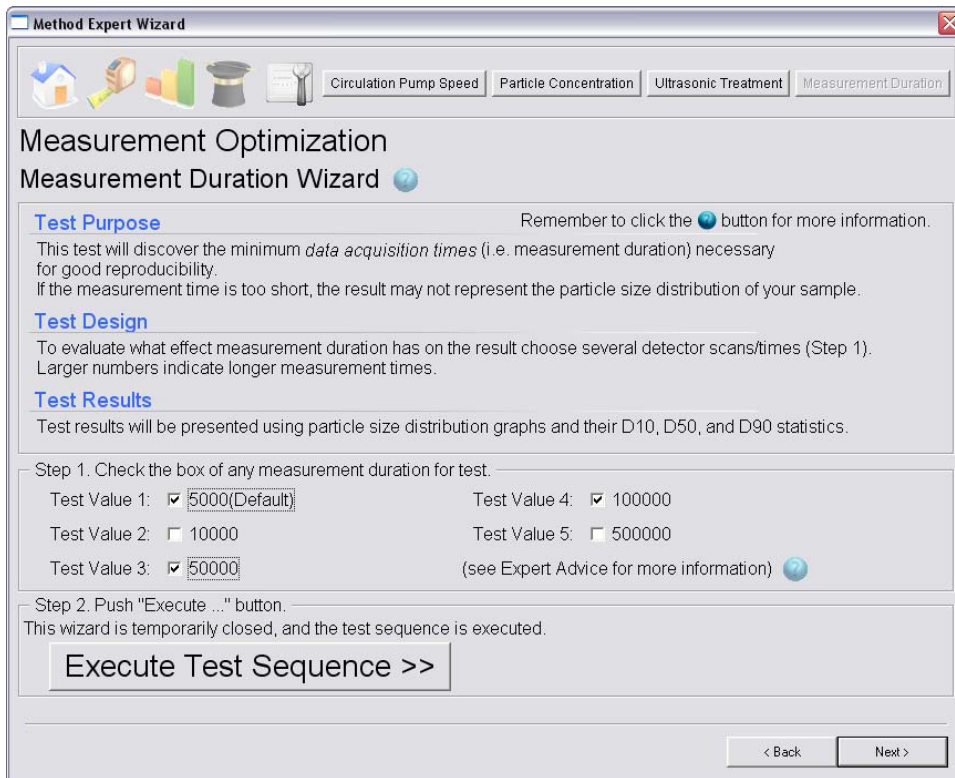
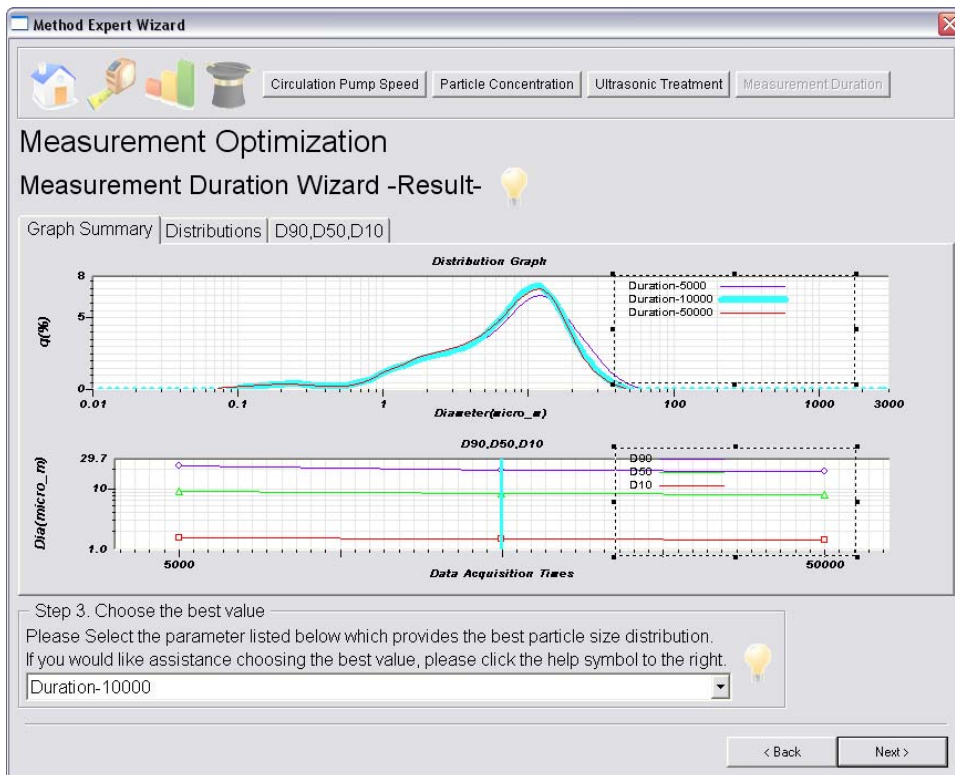*Figure 10: Setup screen for the Measurement Duration Wizard*

Figure 11: Result screen for the Measurement Duration Wizard

Next Test Reproducibility

After completing the method development steps using the Method Expert software an automatic sequence file is created that includes all of the selections made along the way. This sequence file can then be used to analyze this sample using all of the optimized instrument settings. Before using the new method the user should first test the reproducibility of the method in order to confirm result consistency. Several guidelines exist for setting expectations for result reproducibility (3,4). Both the ISO and USP guidelines direct the user to make three independent measurements (prepare sample, measure, drain, repeated three times) and calculate the coefficient of variation (COV) at the d10, d50, and d90. The pass/fail criteria for the COV calculations are slightly different as shown in Table 1.

COV Limits	d10	d50	d90
d50>10 µm			
ISO13320	5%	3%	5%
USP<429>	15%	10%	15%
d50<10 µm			
ISO13320	10%	6%	10%
USP<429>	30%	20%	30%

Table 1: COV limits for reproducibility

The COV calculations and pass/fail criteria based on either ISO13320 or USP<429> can be automatically calculated and displayed in the LA-950/960 software. Figures 12 and 13 show the calculation set up screen and an example of results that pass the selected criteria.

Figure 12: Setup screen for automatic COV calculation and pass/fail criteria

Figure 13: The COV of three unique measurements is calculated and passes the reproducibility criteria

8. TN162 Effect of Concentration on Laser Diffraction Measurements, available from www.horiba.com/us/particle

Summary

Students are not typically trained how to perform particle size analysis while in university. The vast majority of scientists using laser diffraction has either no background or only limited industrial experience and rarely has received formal training. Creating robust, reproducible methods for new samples using laser diffraction requires detailed understanding of each instrumental condition that can affect results. This information can be transferred through training by the vendor, but there are typically more users of a system than resources for extensive training. The solution to this conundrum lies in creating expert software that brings the required knowledge directly to the user. The Method Expert software module encapsulates the knowledge required to create expert level new methods and makes every user capable of generating the best possible data.

References

1. T. Allen, Particle Size Measurement, Chapman and Hall, 4th Edition, 1993
2. ISO 14488 Particulate materials - Sampling and sample splitting for the determination of particulate properties, available from http://webstore.ansi.org/
3. ISO 13320-1 Particle size analysis – Laser diffraction methods, available from http://webstore.ansi.org/
4. USP<429> Light Diffraction Measurement of Particle Size
5. For sample splitting devices see http://www.retsch.com or http://www.quantachrome.com
6. ISO 14887 Sample preparation – Dispersing procedures for powders in liquids, available from http://webstore.ansi.org/
7. TN153 Understanding the Chi Square and Residual R Parameter, available from www.horiba.com/us/particle

9755 Research Drive
Irvine, CA 92618 USA
1-800-446-7422
www.horiba.com/us/particle
labinfo@horiba.com