1. "Cycle" button and clicking "New Sample" button in the Run Cycle dialogue box gives

Universal Liquid Module Run Cycle 🛛 🔀					
New Sample 🔲 Repeat (	Start				
Clear All					
🔽 Auto Rinse	14:43 27 Oct 2009	Options Cancel			
🔲 De-bubble	11:14 Today				
Measure Offsets	11:16 Today				
🗹 Align	11:17 Today				
Measure Background	11:18 Today				
🔽 Measure Loading	(by Standard Obscuration)	Sonicate during Loading			
Enter Sample Info	Sample Info	Sonicate Power 📔 💌			
Enter Run Settings	Run Settings				
Start 3 Runs	Save Export Print				
Auto Rinse					

2. "Options" button gives: measure offsets, align laser, measure background time, show reference background, number of rinses, rinse time, load by standard obscuration.

(Remember ~ rule of 60s for offsets, alignment, and background.) Try to rinse once with organic solvents to cut down on waste.

Reference background vs. new background shows if instrument is clean enough.

Universal Liquid Module Run Cycle Opti	ons 🛛 🔀
Run Control Sample     Every     1     Comment:	Repeat Cycle Defaults OK Color Cancel
Measure Offsets for 60 seconds Every 60 minutes Measure Alignment C Manual Every 60 minutes	Sample Loading Options By standard Obscuration By Obscuration 10 % By PIDS Obscuration 45 % By Sample Weight 0 grams 0 By Sample Volume
Measure Backgrounds for 60 seconds ✓ Show Reference Background C:\LS13320\calfiles\ZUBK7774.\$LS New Reference Background	Enable Auto-Dilution         Auto Rinse:       Drain for         10       seconds         Repeat       2         times

3. "Run Settings" pulls up the run settings dialogue box.

Universal Liquid Module Run Setti	ngs		
Diffraction and PIDS	Pump Speed	• (Off)	эк
Before first run Sonicate for seconds	Wait for 10 se	conds	ancel
Run length: 90 seconds 🕅 9	ionicate during run	Sonicate Power: 1	~
Number of runs: 🛛 🗌 Save Aver	age of All Runs 🛛 🗍	Print Average Repo	ort
Between runs			
Sonicate for seconds	Wait for 10 se	conds	
After each run			
Compute Sizes Na During Run	anomillAdiMatt.rf780d	Model	
🔽 Save File	<f60>_<r2>.<x></x></r2></f60>	File Name	
🔲 Export Data	Tab delimited (.xls)	Export	
Print Report	PDFCreator	Report	
S:\M	att Batt\LS\10-28-09	Folder	

- a. Pump Speed: sets stir speed from ~ 270 rpm to 2700 rpm. Keep pump speed low to prevent bubble formation.
- b. Sonicate: No sonicator so leave Sonicate boxes empty.
- c. Wait For: Time to wait before collecting data.
- d. Run Length = data collection time; 60 seconds minimum for PIDS.
- e. Number of Runs: Number of times sample analyzed
- f. Compute Sizes, Save File.
- g. Folder: select data path in LS Data folder or elsewhere.
- h. Model: Select optical model. "Model" is a set of refractive indices, real and complex, ideally at all four wavelengths of the LS, used to calculate the scattering intensity of a single particle, for a set of diameters, and scattering angles. (See below)

Below are the Select Optical Model dialogue box, an "extended" optical model including the PIDS wave lengths, and a simple optical model for the laser wavelength of 780 nm. The web site of Filmetrics, <u>http://www.filmetrics.com/refractive-index-database</u>, has real and complex indices for a large number of materials, measured as thin films.

Select Optical Model		Optical Model				Optical Model			X
Water 1.332 Optical Model	OK Cancel Get Info New Model	silver.ff780d 12:39 28 Jul 2009 Fluid R.I. Real Laser: 1.332 FIDS 450 nm: 1.332	Sam Real 0.143 0.151	ple R.I. Imaginary 5.1 2.5	OK	garnet.rf780d 9:21 5 Mar 2003 garnet in water Fluid R.I.	Sar	nple R.I.	OK
NanomillAdiMatt.rf780d	e-factor: 1	600 nm: 1.332	0.124	3.7		Real	Real	Imaginary	
✓ Include PIDS data		900 nm: 1.332	0.17	6.1		1.333	1.8	0.3	

When the refractive indices,  $n(\lambda_k)_{Re}$ ,  $n(\lambda_k)_{Im}$  are entered, the scattering intensities,

$$i(\theta_m, d_a, n(\lambda_k)_{Re}, n(\lambda_k)_{Im})$$

of a single particle of diameter  $d_a$  at scattering angle  $\theta_m$ , and wavelength  $\lambda_k$  are *calculated for a fixed set of diameters*, *scattering angles*, *and wavelengths*. There are 115 diameters, 136 angles, and 4 wavelengths used.

The total scattering intensity at each detector at scattering angle  $\theta$  is the scattering intensity of a single particle,  $i(\theta_m, d_a, n(\lambda_k)_{Re}, n(\lambda_k)_{Im})$ , times the number of particles  $N(d_a)$  summed over all diameters and wavelengths:

$$I(\theta_m) = \sum_{a=1}^{A} \sum_{k=1}^{4} i(\theta_m, d_a, n(\lambda_k)_{Re}, n(\lambda_k)_{Im}) * N(d_a) = \sum_{d} i(\theta_n, d) n(d)$$

This can be written in matrix form

$$\begin{bmatrix} I(\theta_1) \\ \vdots \\ I(\theta_n) \end{bmatrix} = \begin{bmatrix} i(\theta_1, d_1) & \dots & i(\theta_1, d_A) \\ \vdots & \dots & \vdots \\ i(\theta_M, d_1) & \dots & i(\theta_M, d_A) \end{bmatrix} \begin{bmatrix} N(d_1) \\ \vdots \\ N(d_A) \end{bmatrix} \Rightarrow$$

Simply,  $I = iN \Rightarrow N = i^{-1}I \Rightarrow N \sim I/i$ . Therefore, if *i* is too large  $\sim \Rightarrow N$  too small, *i* small $\sim \Rightarrow N$  large.

The indvidual scattering intensities,  $i(\theta_m, d_a, n(\lambda_k)_{Re}, n(\lambda_k)_{Im})$  are calculated from the Mie solution to Maxwell's equations for the interaction of electromagnetic plane waves with a spherical object. It is too difficult to calculate if the diameter is too much larger than the wavelength, so the intensities are calculated from Fraunhofer diffraction theory. Fraunhofer does not depend on optical properties. If your particles are greater than **10** µm, the results of your measurement will not be too sensitive to changes in optical model. Below **1**µm, the results can be very sensitive to the correct, extended optical model, so beware in your interpretation of the results, if you do not have a reliable set of refractive indices. The interpretation of scattering intensities as a function of angle also assumes particles are, homogeneous in material, and isotropic in refractive response. The software does not know how to interpret scattering patterns from complex particles in terms of particle size distribution. Only simple spherical particles conform to the assumptions of the Mie solution. 4. Sample Info: This is where your file names will be created.

Enter Sample	: Info		$\mathbf{X}$
File ID: S	ample Name	Operator: Your N	ame
Sample ID: S	ample Name 2	Bar Code:	
Comment 1:		Run Number: 1	
Comment 2:			
Sample Density	n 0 g/mL	Control Sample	Sample Statistics
Fluid: w	vater		Select Fluid
Template: < File Name: S	F60>_ <r2>.<x> Sample Name_01.\$Is</x></r2>		Clear All
File Nan	ne		Cancel OK

Fields in the sample info box to be used as file names are selected from the File Name Generation dialogue check box. Run number increases by **1** after every run.

File Name Generation		
✓ <f#> File ID Up to 60 characters</f#>	Sample ID Up to <a href="https://www.up.to">O</a> characters	OK Cancel
C <0#> Operator	       	Clear All
CP > Date (28 Oct 2009)	<pre>T#&gt; Time 2: 13-38 </pre>	Defaults
R#> Run Number     2   digits	C <u#> Unique Number</u#>	
<>> Instrument Extension LS: \$Is		
Example: <f60>_<r2>.&lt;&gt;&gt;</r2></f60>		
Use Example <1 Template: <a href="https://www.completencemplatescore">             Use Example &lt;1         </a> Template: <a href="https://www.completencemplatescore">             Vise Example </a> Template: <a href="https://www.completencemplatescore">                  Vise Example <a href="https://www.completencemplatescore">                   Vise Example <a href="https://www.completencemplatescore"></a></a></a>	T#> or <u#> recommended</u#>	

- 5. Press "Start" in the run cycle dialogue box.
- 6. Measure Loading: When software gets to "measure loading", add particles until the value for "Obscuration" is between 8 and 12 % and the PIDS is between 40 to 55%. Keep both to the high end of their range, without obscuration going over 12%, or PIDS over 55%.
- 7. Repeat the cycle, new sample, sample info, OK, Start for each new sample.
- 8. Change run settings for new sample types as needed.