

Protein Crystallization Application

The Malvern Zetasizer Nano (Malvern.com)
Cusop Technology (Cusoptechnology.com)

Sample Details

During a visit to a Boston Pharmaceutical Company to test the Spinvette under client conditions, our customer provided us with a protein candidate that has been prepared for crystallization. While measuring the sample using the Spinvette and a Helma 12 uL cuvette, the computer malfunctioned and we lost some beautiful homogenous data for both the Helma cuvette and the Spinvette. After 20 minutes with the sample and cuvette left inside the Malvern Zetasizer Nano, the computer was replaced and a new analysis was carried out. This particular protein was described by our customer as a finicky behaving protein as sometimes it will form crystals and then other times it will not. The customer was not surprised by the multiple distributions that we obtained in the Helma Cuvette!

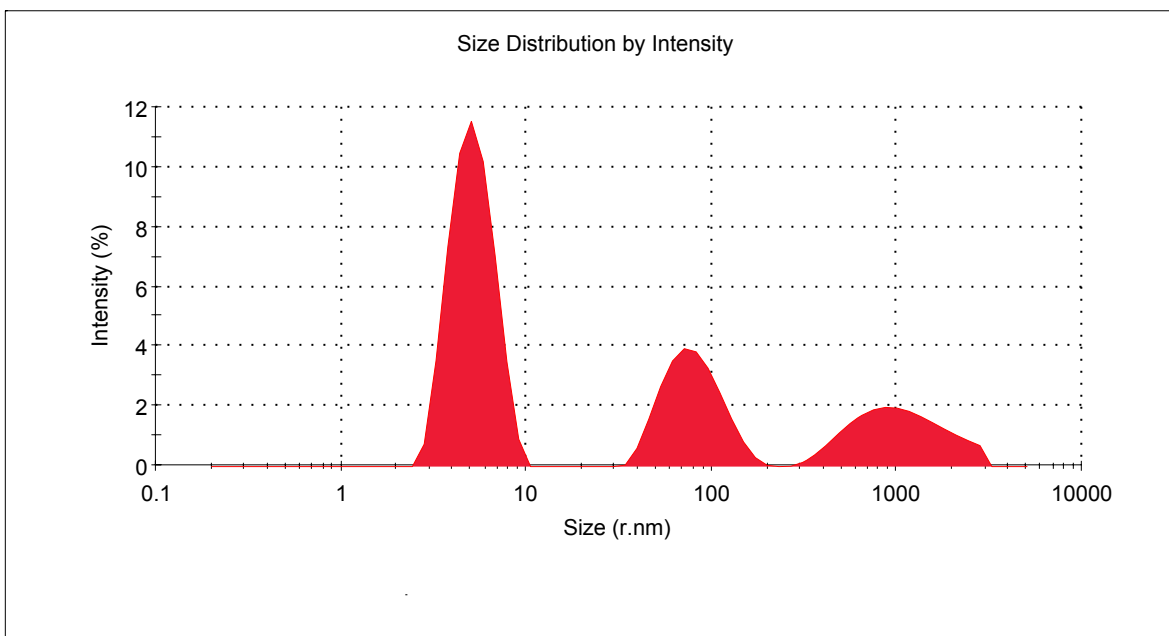
System

Temperature (°C):	25.0	Duration Used (s):	70
Count Rate (kcps):	207.2	Measurement Position (mm):	4.20
Cell Description:	Low volume glass cuvette (12µL)	Attenuator:	9

Results

	Size (r.nm):	% Intensity	Width (r.nm):
Z-Average (r.nm): 10.17	Peak 1: 5.214	55.2	1.348
Pdl: 0.550	Peak 2: 81.58	24.7	28.37
Intercept: 0.956	Peak 3: 1141	20.1	614.9

Result quality : **Refer to quality report**



Helma 12uL Cuvette

Sample Details

We then tested the sample using the Spinvette. As there is not a preset in the Malvern Zetasizer Nano software, Cusop Technology has determined that a setting of 5.5 mm is the ideal position for the NIBS probe. Therefore when setting up the method we recommend our users to choose the Clear Disposable Zeta Cell for cell choice as this defaults to a 5.5 mm position

Again we get very similar results to the Helma 12 uL quartz cuvette, the count rate and the attenuation is very are almost identical between the two runs. But in both cases there is **QUALITY REPORT WARNING**, the correlation functions for this aggregating sample are complex and sedimentation is beginning to occur.

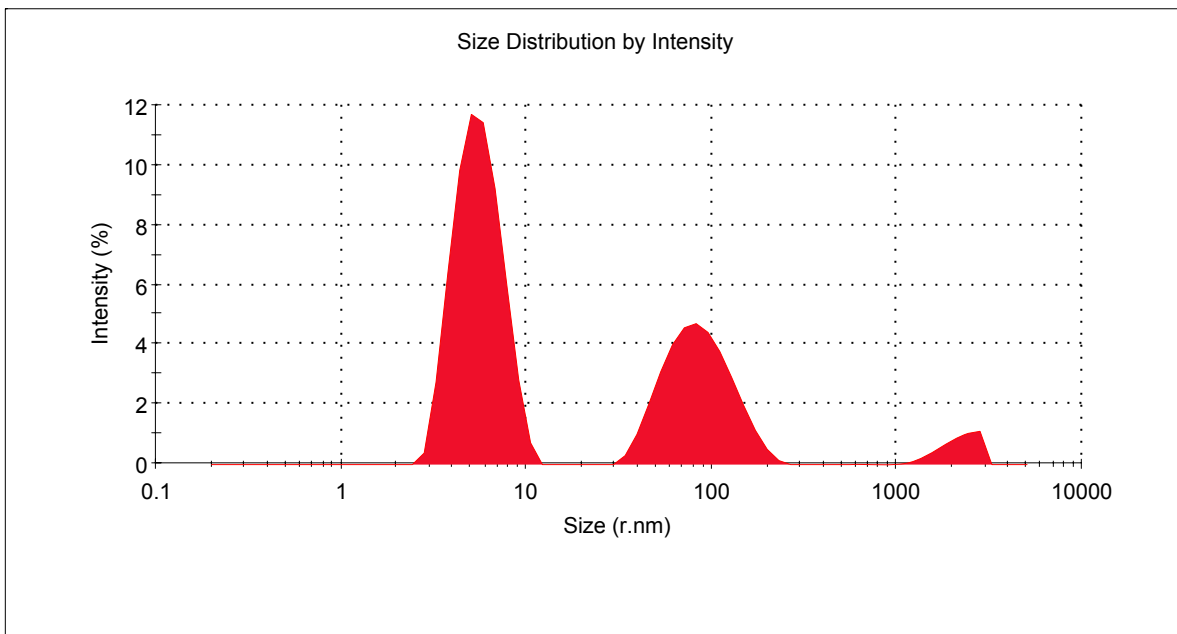
System

Temperature (°C): 24.9	Duration Used (s): 60
Count Rate (kcps): 195.1	Measurement Position (mm): 5.50
Cell Description: Clear disposable zeta cell	Attenuator: 9

Results

	Size (r.nm):	% Intensity	Width (r.nm):
Z-Average (r.nm): 8.816	Peak 1: 5.642	61.1	1.584
Pdl: 0.549	Peak 2: 89.30	34.5	36.24
Intercept: 0.851	Peak 3: 2190	4.4	465.3

Result quality : Refer to quality report



Spinvette Data

We removed the Spinvette from the Malvern Zetasizer Nano and placed it into an Eppendorf microfuge and the protein sample was spun for 5 minutes at 14,000 g. The Spinvette was placed back into the Malvern Zetasizer Nano and another set of runs were started. From the new set of results it is obvious that the sedimented material has been removed from the illuminated sample volume and the system states that a GOOD RESULT QUALITY has been obtained. And the count rate also decreased to 132 kcps. Now it is possible to track the aggregation process of the main peak as function of time.

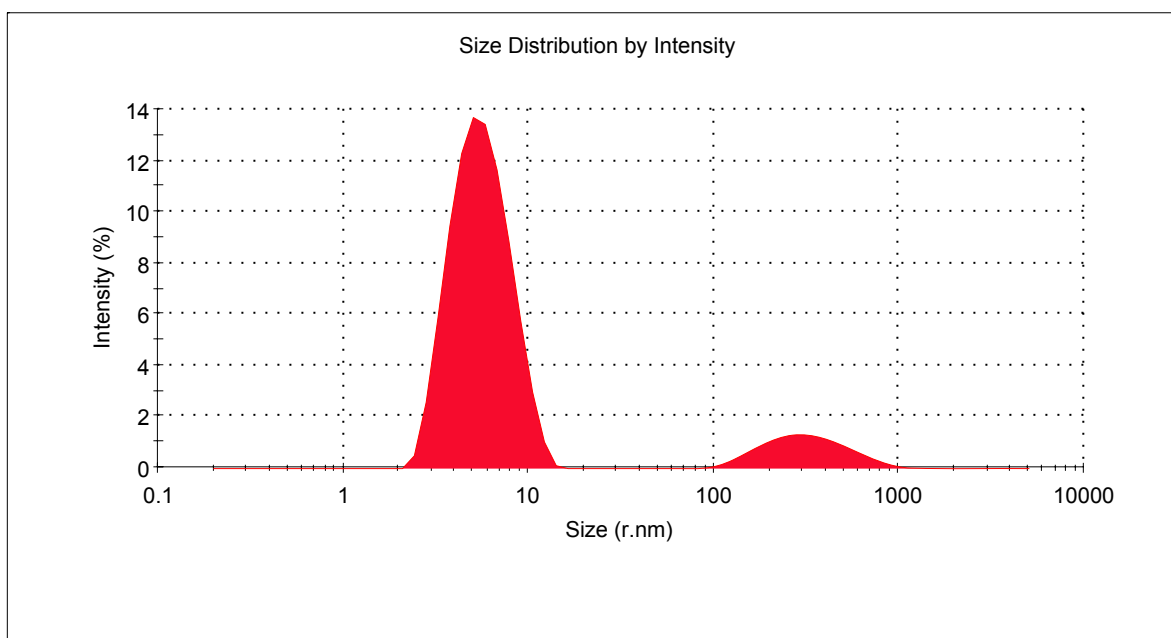
The Spinvette has equal optical quality to the Quartz 12 uL cuvette (list price \$567.00), The Spinvette is rated currently to a minimum volume of 25uL A Spinvette kit costs \$850, but you get 10 high quality Spinvettes and a holder designed for the Malvern system, with the future knowledge that your replacement cost for further Spinvette could be as low as \$3.50 a Spinvette at 200 quantities. If you currently filter your samples prior to analysis, a case of Anotop (0.02 um) 010 filters will cost approx \$209 dollars for 50 filters. That is just over \$4 per filter. Therefore the Spinvette is actually more cost effective as it can be used in the sample preparation instead of filtration. Sample recovery is much higher, reliable and convenient then using a combination of filters with a disposable cuvette or quartz cuvette. With a Spinvette there is no sample loss. You can store the Spinvette with out having to remove sample. Also the act of quickly centrifuging small volumes of sample removes bubbles that often occur during sample transfer. Depending on rotor size of the microfuge, samples can be prepared simultaneously up to 24 samples at a time.

System

Temperature (°C):	25.1	Duration Used (s):	70
Count Rate (kcps):	139.9	Measurement Position (mm):	5.50
Cell Description:	Clear disposable zeta cell	Attenuator:	9

Results

	Size (r.nm):	% Intensity	Width (r.nm):
Z-Average (r.nm): 5.782	Peak 1: 5.789	88.0	2.037
Pdl: 0.277	Peak 2: 343.3	12.0	174.8
Intercept: 0.837	Peak 3: 0.000	0.0	0.000
Result quality : Good			



Spinvette Spun at 14,000 g