HORIBA Scientific

Technical Note

ViewSizer 3000 Repeatability and Reproducibility

TN182

ViewSizer 3000 Repeatability and Reproducibility

Introduction

Repeatability and reproducibility are important for understanding the uncertainty in measurement results. Here "repeatability" refers to placing a sample in the analyzer and repeating the measurement without removing the sample. With a stable sample, this can be a pure test of instrument stability. "Reproducibility" is divided into two tests. The first, "in the same vial" refers to preparing a parent suspension and then refilling the instrument sample cell between measurements. Thus reproducibility also includes the effects of sample handling. The second, "different vials" refers to preparing new suspensions and measuring. This reproducibility then includes the variations in sample preparation.

As suggested above, like many particle analyzers, the repeatability and reproducibility of the ViewSizer depend on using a sufficiently well-behaved sample. In addition, reproducibility also depends on the details of sample preparation.

This document presents repeatability and reproducibility results with the ViewSizer 3000. It also includes experimental details that should help the analyst achieve similar results. In particular, note the change in diluent for different sample types along with the attention to diluent cleanliness.

Methods

Each sample was measured by the following method:

1. 100nm PSL

- Prepare dilution liquid of 1mM SDS in XZero nanopure water, filtered through a 20nm filter.
- Dilute 100nm PSL 400,000x times in filtered 1mMS SDS solution by serial dilution of no more than 100x dilution for each step.
- Add diluted sample to cuvette and insert cuvette into instrument.
- Adjust focus to attain sharp images on particles.
- Set Blue laser to 70mW, Green laser to 12mW, and Red laser to 8mW.
- Set Gain to 24 and Exposure to 15.
- Take a measurement of 25 videos.
- Take 8 more measurements of 25 videos each on the same sampling.
- Measure 2 new samplings from the same vial to test reproducibility from same vial.
- Prepare 2 new vials of diluted sample and take 1 measurement from each vial to test reproducibility from different vials.

2. 400nm PSL

- Prepare dilution liquid of 1mM SDS in XZero nanopure water, filtered through a 20nm filter.
- Dilute 400nm PSL 6,000x times in filtered 1mMS SDS solution by serial dilution of no more than 100x dilution for each step.
- Add diluted sample to cuvette and insert cuvette into instrument.
- Adjust focus to attain sharp images on particles.
- Set Blue laser to 70mW, Green laser to 12mW, and Red laser to 8mW.
- Set Gain to 24 and Exposure to 15.
- Take a measurement of 25 videos.
- Measure 2 new samplings from the same vial to test reproducibility from same vial.
- Prepare 2 new vials of diluted sample and take 1 measurement from each vial to test reproducibility from different vials.

3. Fuso PL-7

- Dilute the sample 2,000,000x times in XZero nanopure water.
- Add diluted sample to cuvette and insert cuvette into instrument.
- Adjust focus to attain sharp images on particles.
- Set Blue laser to 70mW, Green laser to 12mW, and Red laser to 8mW.
- Set Gain to 24 and Exposure to 15.
- Take a measurement of 25 videos.
- Take 14 more measurements of 25 videos each.

Results and Discussion

Repeatability and reproducibility data are tabulated in Tables 1–6. The coefficient of variation, CoV (as standard deviation over mean) increases with increasing complexity. For 100 nm PSL, simply measuring over and over gives a CoV of 2.35%. However, this rises to almost 6% when fresh samples are prepared.

In addition, and not apparent in these tables is the concern about sample drift. If the sample changes over time, this will appear as a higher CoV (and as a trend in the data). For fast measurements, this issue may be small. But the large number of repeats (15) for the PL-7 sample in Table 6 takes over an hour to collect and a sample that changes quickly would not show similarly good results.

For Poisson statistics, one expects the repeatability (as the standard deviation) to be the square root of the number of particles counted. In table 7, these values are compared and the observed repeatability is close to expectations.

As an example, measured distributions of PL-7 are overlaid in Figure 1.

No.	Counts (50 - 150nm)	D50 (nm)	Concentration (counts/mL)
1	2656	100.19	1.700E+13
2	2673	100.52	1.711E+13
3	2714	100.54	1.737E+13
4	2575	102.4	1.648E+13
5	2565	101.25	1.642E+13
6	2747	101.36	1.758E+13
7	2607	101.63	1.668E+13
8	2669	101.21	1.708E+13
9	2607	100.83	1.668E+13
Average	2646	101.10	1.693E+13
SD	62	0.67	3.973E+11
CoV	2.35%	0.67%	2.35%

Table 1: 100nm PSL Repeatability.

No.	Counts (50-150nm)	D50 (nm)	Concentration (counts/mL)
1	2705	99.25	1.731E+13
2	2778	101.02	1.778E+13
3	2948	98.93	1.887E+13
Average	2810	99.73	1.799E+13
SD	125	1.13	7.980E+11
CoV	4.44%	1.13%	4.44%

Table 2: 100nm PSL Reproducibility from same vial.

No.	Counts (50-150nm)	D50 (nm)	Concentration (counts/mL)
1	2656	100.19	1.700E+13
2	2705	99.25	1.731E+13
3	2963	97.95	1.896E+13
Average	2775	99.13	1.776E+13
SD	165	1.12	1.056E+12
CoV	5.94%	1.13%	5.94%

Table 3: 100nm PSL Reproducibility from different vials.

No.	Counts (200-600nm)	D50 (nm)	Concentration (counts/mL)
1	3106	382.07	2.982E+11
2	3173	379.27	3.046E+11
3	3149	379.65	3.023E+11
Average	3143	380.33	3.017E+11
SD	34	1.52	3.259E+09
CoV	1.08%	0.40%	1.08%

Table 4: 400nm PSL Reproducibility from same vial.

No.	Counts (200-600nm)	D50 (nm)	Concentration (counts/mL)
1	3106	382.07	2.982E+11
2	3142	384.31	3.016E+11
3	2961	382.9	2.843E+11
Average	3070	383.09	2.947E+11
SD	96	1.13	9.198E+09
CoV	3.12%	0.30%	3.12%

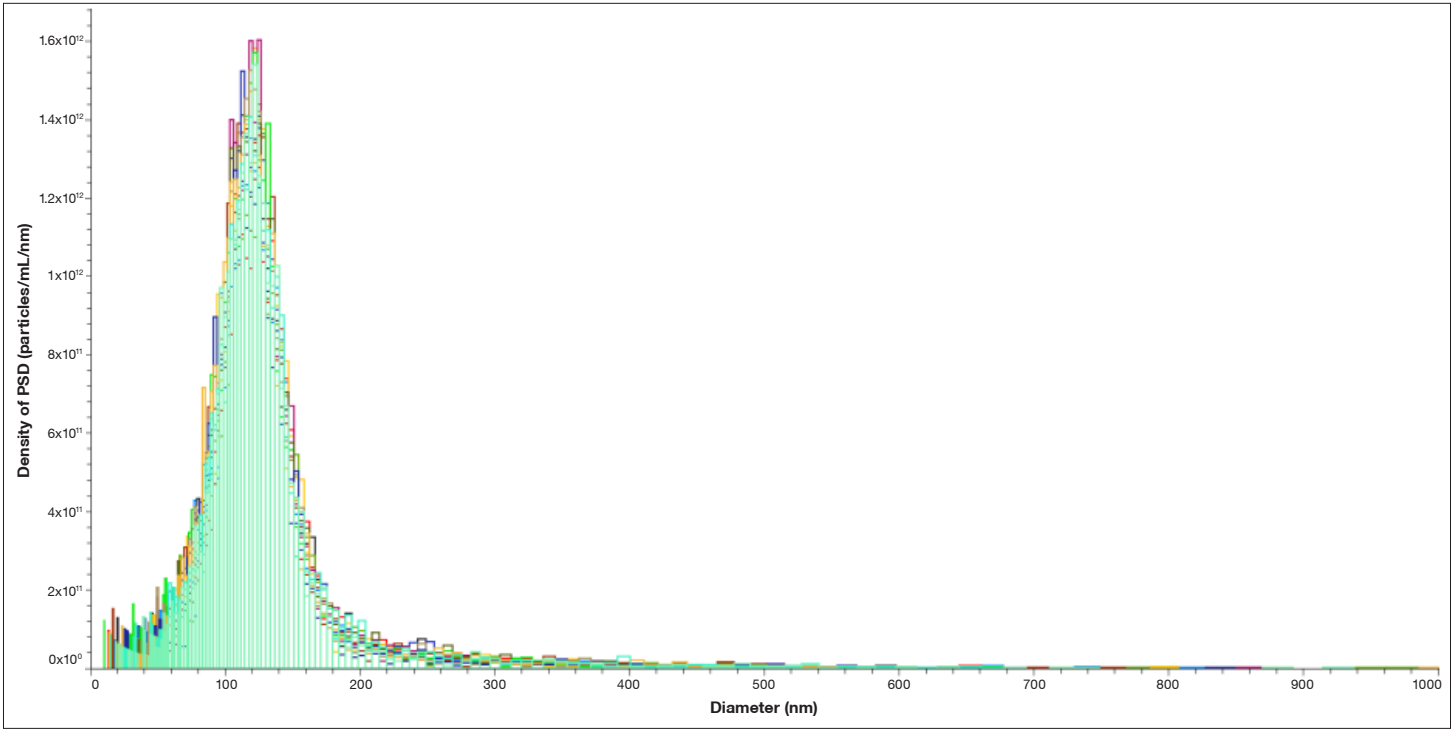
Table 5: 400nm PSL Reproducibility from different vials.

No.	Counts	D50 (nm)	Concentration (counts/mL)
1	2565	121.41	8.210E+13
2	2473	123.41	7.910E+13
3	2677	121.21	8.570E+13
4	2627	123.6	8.410E+13
5	2860	121.58	9.150E+13
6	2721	120.57	8.710E+13
7	2596	120.56	8.310E+13
8	2726	120.73	8.720E+13
9	2715	119.87	8.690E+13
10	2691	119.95	8.610E+13
11	2701	120.64	8.640E+13
12	2494	119.59	7.980E+13
13	2685	118.66	8.590E+13
14	2647	121.41	8.470E+13
15	2627	120.75	8.410E+13
Average	2654	120.93	8.492E+13
SD	97	1.30	3.106E+12
CoV	3.66%	1.08%	3.66%

Table 6: PL-7 Repeatability.

Sample	Average Counts	Expected CoV for Concentration	Measured CoV for Concentration
100 nm PSL	2646	1.94%	2.35%
PL-7	2654	1.94%	3.66%

Table 7: Repeatability of sample concentration compared to expectation. The expectation is 1/sqrt (number of particles counted) for Poisson statistics. Note that measured repeatability is quite close to the expected value.



Sample	Color	Mode	Total Counts	Mode (nm)	D50 (nm)	D10 (nm)	D90 (nm)	Span (nm)	Concentration (particles/mL)
PL-7_01		MPTA	2565	119.22	121.41	84.80	168.14	0.690	8.21×10^{13}
PL-7_02		MPTA	2473	125.33	123.41	87.91	174.85	0.700	7.91×10^{13}
PL-7_03		MPTA	2677	113.40	121.21	86.30	170.83	0.700	8.57×10^{13}
PL-7_04		MPTA	2627	125.33	123.60	88.48	176.56	0.710	8.41×10^{13}
PL-7_05		MPTA	2860	122.24	121.58	87.00	169.40	0.680	9.15×10^{13}
PL-7_06		MPTA	2721	119.22	120.57	87.14	166.78	0.660	8.71×10^{13}
PL-7_07		MPTA	2596	113.40	120.56	85.83	168.38	0.680	8.31×10^{13}
PL-7_08		MPTA	2726	125.33	120.73	88.09	170.98	0.690	8.72×10^{13}
PL-7_09		MPTA	2715	122.24	119.87	84.08	164.75	0.670	8.69×10^{13}
PL-7_10		MPTA	2691	119.22	119.95	85.76	166.48	0.670	8.61×10^{13}
PL-7_11		MPTA	2701	125.33	120.64	85.78	170.65	0.700	8.64×10^{13}
PL-7_12		MPTA	2494	107.87	119.59	85.79	163.20	0.650	7.98×10^{13}
PL-7_13		MPTA	2685	116.27	118.66	84.23	165.00	0.680	8.59×10^{13}
PL-7_14		MPTA	2647	119.22	121.41	86.43	178.69	0.760	8.47×10^{13}
PL-7_15		MPTA	2627	122.24	120.75	87.22	169.05	0.680	8.41×10^{13}
		Average	2653.67	119.72	120.93	86.32	169.58	0.69	8.49×10^{13}
		CoV (%)	3.54	4.19	1.04	1.50	2.50	3.61	3.54

Figure 1: Overlay of size distribution of 15 runs of PL-7 with the ViewSizer 3000.

Conclusion

Repeatability of 2~3% can be achieved with the ViewSizer 3000.