Evaluation of Performance Across Multiple CytScop® Pro

Introduction

The CytScop® Pro Intelligent Cell Analyzer demonstrates excellent precision within the concentration range of 0.2M-10M. In practical applications where multiple devices are used simultaneously, especially when measuring the same batch of samples, it is important to determine whether there are any deviations in the measurement results between multiple CytScop® Pro units and whether these deviations meet the performance technical specifications.

Furthermore, when the CytScop® Pro Intelligent Cell Analyzer is utilized for production quality control analysis and in GMP production environments, it is imperative that the comparison of data between instruments falls within an acceptable range. Additionally, the data deviations between instruments are often used to assess the intermediate precision of the instruments.

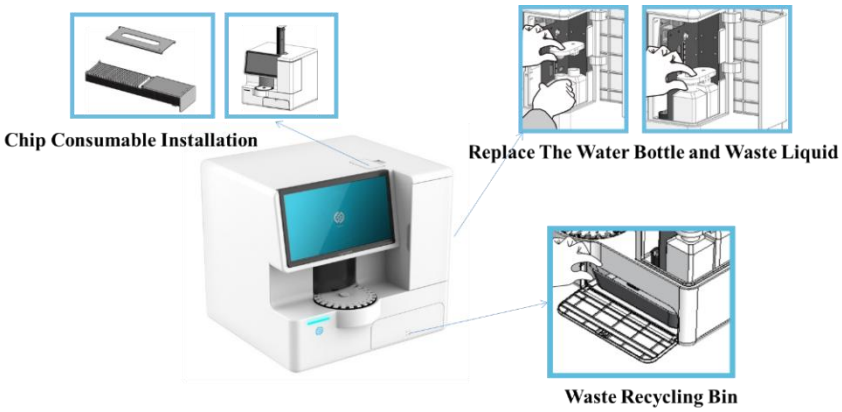

Figure 1. CytScop® Pro

Methods

By conducting a series of serial dilutions, samples with different dilution concentrations are obtained. Subsequently, each diluted sample is tested on three randomly selected CytScop® Pro units, with repeated testing of the same samples. Finally, the performance of the instrument is evaluated through a series of data analyses.

Following the dilution guidelines provided by ISO 6887-1:2017, the original sample solution is serially diluted to obtain six dilution gradients: 1, 0.5, 0.25, 0.125, 0.0625, 0.03125. Nine parallel samples are taken at each concentration gradient, and each instrument tests three parallel samples at each concentration.

Dilution	Nominal Concentration ($\times 10^6$)/mL
1	10

0.5	5
0.25	2.5
0.125	1.25
0.0625	0.625
0.03125	0.3125

Samples and instrument settings

Cell Sample

CHO cells, short for Chinese Hamster Ovary cells, are a commonly used mammalian cell line in biotechnology and biopharmaceutical research. Percentage viability and total Jurkat cell concentration are 98% and 10×10^6 cells /mL.

Test Mode

AOPI stain

Instrument Settings

Cell type	AOPI-CHO
Number of sampling areas	3
Settling time (s)	40
Number of mixes	3
Minimum cell diameter	8
Maximum cell diameter	24
Agglomeration factor	0.8
Minimum fluorescent area	15
AO fluorescence filtration coefficient	4
PI fluorescence filtration coefficient	15
BF/FL (mu s)	8000/300000
Gain	0

Results

Coefficient of Variation (C.V) is a statistical measure used to quantify the degree of variation in a set of observations. It is commonly employed to reflect the dispersion of a dataset and indicate the measurement precision of an instrument. Table 1 below illustrates the variation in cell concentration measurements and their corresponding C.V values across three CytScop® Pro units, while Figure 2 provides a visual representation of how the C.V values change across different concentrations for these three instruments.

Table 1 shows the measurement of each dilution concentration in 3 instruments

Instrument	Dilution	Average concentration(x 10^6) cells/mL	C.V of concentration cells/mL	Samples
1	1	10.91	1.15%	3
	0.5	5.66	1.46%	3
	0.25	2.89	2.54%	3
	0.125	1.44	1.18%	3
	0.0625	0.76	2.15%	3
	0.03125	0.39	2.44%	3
2	1	11.19	2.42%	3
	0.5	5.76	1.73%	3
	0.25	2.79	1.88%	3
	0.125	1.46	2.89%	3
	0.0625	0.76	0.51%	3
	0.03125	0.40	2.74%	3
3	1	10.92	1.27%	3
	0.5	5.65	2.19%	3
	0.25	3.15	2.50%	3
	0.125	1.5	1.63%	3
	0.0625	0.84	0.56%	3
	0.03125	0.41	2.32%	3

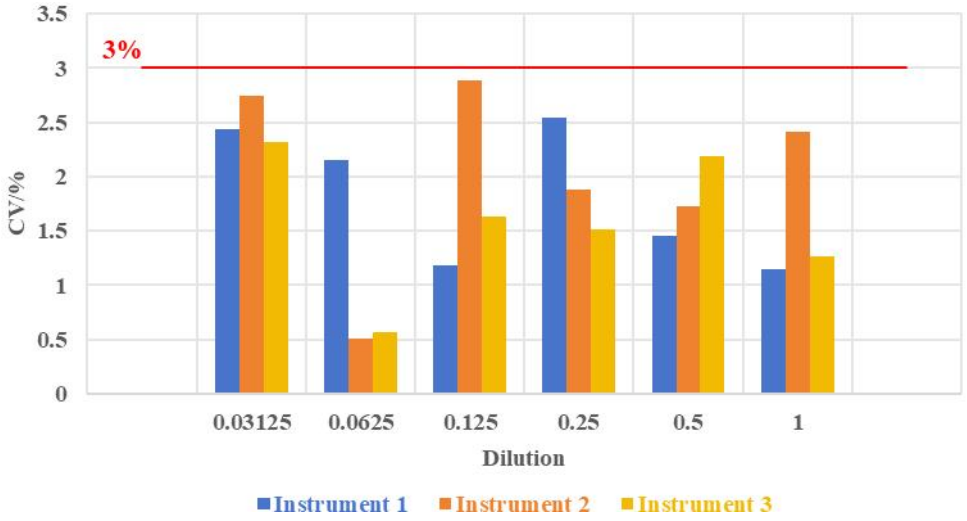

Figure 2. The variation of the coefficient of variation (C.V) in concentration measurements.

Linear regression analysis was conducted separately on the test results of the three CytScop® Pro

instruments, and the results are shown in Figure 3.

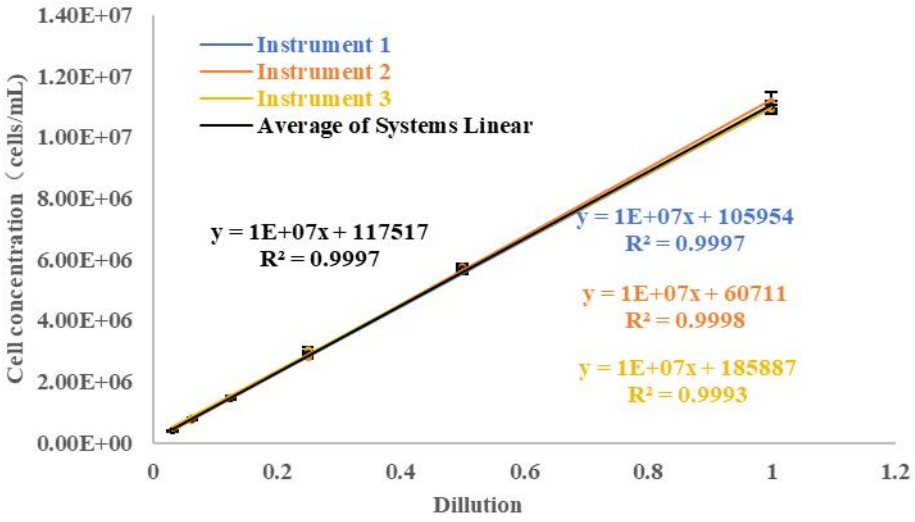

Figure 3. The linear correlation between the three instruments under serial dilution.

Statistical analysis was performed on the total cell concentration and viable cell concentration of the three CytScop® Pro tests at various dilution gradients, with the results shown in Figures 4 and 5.

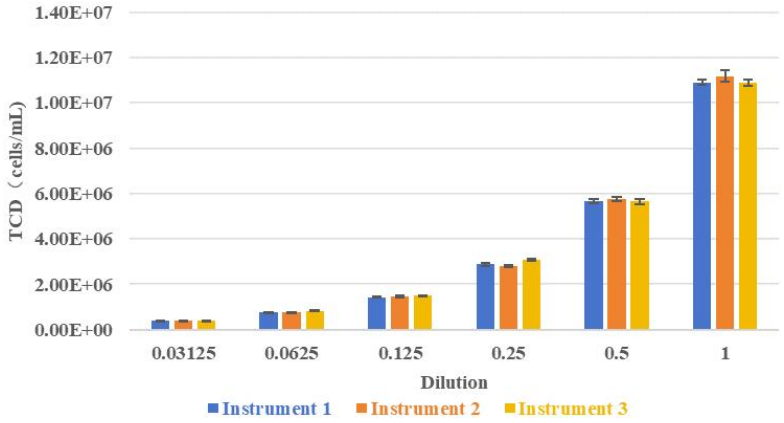

Figure 4. Comparison of total cell concentration measurements by the three instruments.

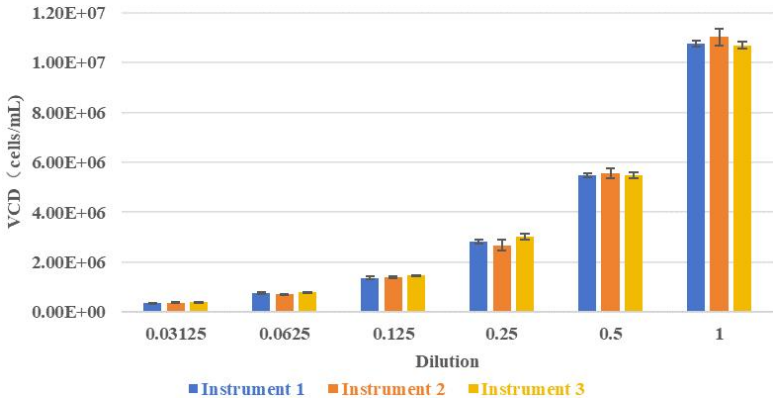

Figure 5. Comparison of viable cell concentration measurements by the three instruments.

Analysis of the diameters measured by the three CytScop® Pro instruments at each concentration gradient, with the results shown in Figure 6.

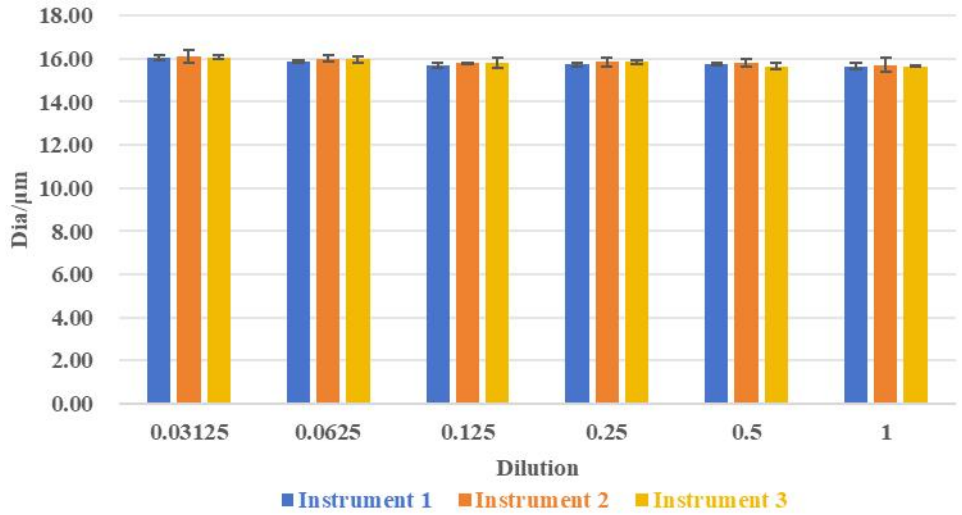

Figure 6. Comparison of diameter measurements by the three instruments.

Analysis of the viability measured by the three CytScop® Pro instruments at each concentration gradient, with the results shown in Figure 7.

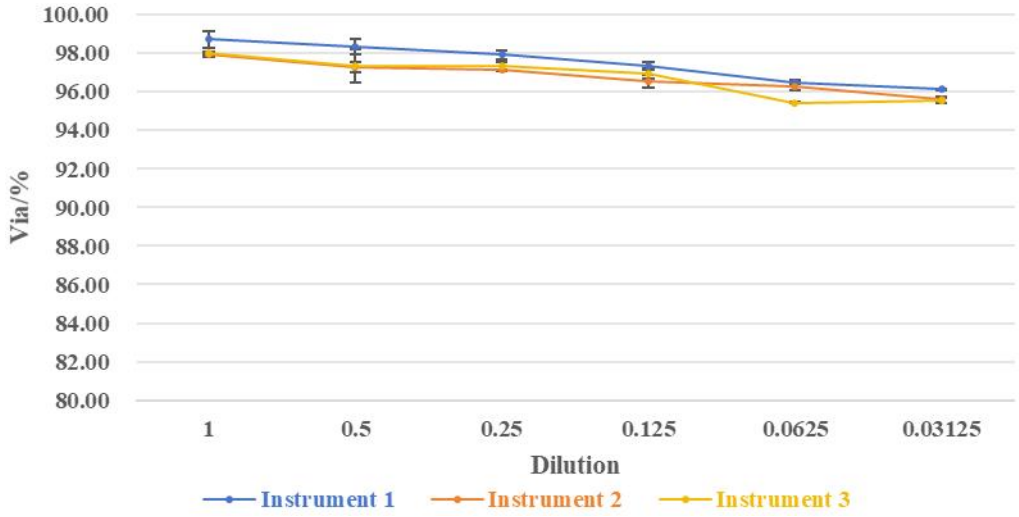

Figure 7. Comparison of cell viability measurements by the three instruments.

Conclusion

Performance evaluation of three randomly selected CytScop® Pro instruments for inter-instrument comparison. Table 1 and Figure 2 show the repeatability between parallel samples measured by the three instruments. It can be observed that within the concentration range of 0.3M-10M, all three instruments

demonstrate excellent measurement repeatability, with Coefficient of Variation (C.V) all within 3%, indicating high intermediate precision of the instruments.

By conducting linear regression analysis on samples from the same batch using the three instruments, as shown in Figure 3, it can be observed that all three instruments exhibit a strong linear correlation ($R^2 > 0.999$). Additionally, the average overall linear correlation of the three instruments is excellent, with an R^2 value of 0.9997.

Under different dilution gradients, one-way ANOVA was conducted on the total cell concentration and viable cell concentration measured by the three instruments. The results indicate that there is no statistically significant difference ($p > 0.05$) in the measurements among the three instruments at various concentrations. As shown in Figures 4 and 5, both total cell concentration and viable cell concentration demonstrate consistent patterns. Additionally, a statistical analysis of the diameter measurements, as depicted in Figure 6, reveals that for each instrument, the variation in diameter measurements across different concentrations is minimal, not exceeding 3%. Analysis of diameter measurements at various dilutions for the three instruments indicates minimal differences between the instruments, with the ANOVA analysis of variance showing no significant discrepancies. In the comparative analysis of the viability of the three instruments at various dilutions (as shown in Figure 7), it is observed that within the concentration range of 0.3M to 10M, the viability remains relatively stable across different dilutions. The variation in viability does not exceed 3% compared to the initial solution, and there are no significant differences in viability measurements among the three instruments.

In conclusion, within the range of 0.3M-10M, the CytScop® Pro Intelligent Cell Analyzer shows that the three instruments exhibit no significant differences in the measurement of various cell counting parameters. This indicates minimal differences between the instruments, demonstrating the excellent intermediate precision of the CytScop® Pro.

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