Cell Counting Performance of CytScop® Pro

Introduction

CytScop® Pro is an advanced intelligent cell analyzer. It integrates leading artificial intelligence (AI) image analysis with wide-field large-view microscopy imaging technology which can complete a fully automatic analysis of various parameters of cell samples. At the same time, it also provides customized intelligent cell analysis services based on different cell types. CytScop® Pro is equipped with microfluidic chip consumables developed and produced independently by Shanghai Bioaces Life Science Co., Ltd. This chip utilizes the industry's pioneering dye embedding technology to achieve automatic cell staining in the microfluidic chip, eliminating the need for manual staining and mix. By leveraging fluid mechanics principles to control the even distribution of cells, it enables rapid automatic counting and analysis of cell samples. CytScop® Pro features a rotating sample tray that can test 24 samples at once and supports repeated sampling.

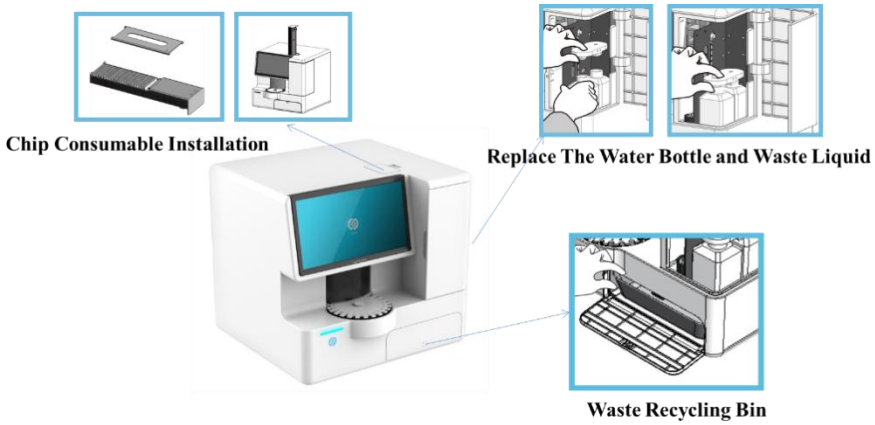

Figure 1. CytScop® Pro

Currently, most of the cell analysis products available on the market use single-channel staining, which often fails to meet the varying requirements for cell counting in experiments. However, this product was designed with dual-channel staining functionality from the outset, compatible with both TB and AOPI staining methods.

During actual testing, there are many factors that can affect the accuracy and precision of cell counting. The accuracy and precision of cell counting results play a crucial role in carrying out subsequent work. Therefore, for cell counters, ensuring that the systematic error of the instrument meets the requirements of standard regulations will be crucial, apart from human operational errors and sample errors.

Methods

To evaluate the cell counting performance of CytScop®Pro, an experimental procedure described in ISO

standards (ISO 6887-1:2017) was employed. This procedure involves assessing the cell counting performance of CytScop®Pro through a series of dilutions.

Different dilutions of samples are obtained through a series of dilutions, and these diluted samples are then subjected to machine testing. The same samples undergo repeated testing, and finally, the instrument performance is evaluated through a series of data analyses.

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

Standard Sample of Microsphere

The nominal value of the standard microspheres is 15μm, 1x10 mL with a concentration of 10×10^6 beads/mL.

Standard Sample Microsphere Instrument Test Parameters

Cell type name: TB- Standard -15μm microspheres

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 97% and 10×10^6 cells /mL.

Instrument Settings

Cell type name	CHO
Number of sampling areas	3
Settling time (s)	30
Number of mixes	3
Minimum cell diameter	8
Maximum cell diameter	24
Agglomeration factor	0.8
Live cell brightness	2
Dead cell brightness	10
Dead cell coefficient	2

BF (μs)	18000
Gain	0

Results

Microsphere Counting Results

The coefficient of variation (C.V) is a statistical measure used to assess the variability of individual observations within a dataset. It is commonly employed to reflect the degree of dispersion in a set of data and can indicate the precision of measurements taken by an instrument. A smaller C.V value suggests that the data from the instrument's tests are less dispersed, indicating better repeatability of the instrument and higher accuracy. As shown in Table 1, the mean C.V for the microsphere sample test data is 1.18%.

Table 1 Original data and calculation results of microsphere experiment

Dilution	0.03125	0.0625	0.125	0.25	0.5	1
	3.55E+05	6.79E+05	1.25E+06	2.82E+06	5.52E+06	10.68E+06
	3.61E+05	6.83E+05	1.29E+06	2.84E+06	5.56E+06	10.71E+06
	3.43E+05	6.86E+05	1.28E+06	2.83E+06	5.59E+06	10.43E+06
TCD Average	3.53E+05	6.83E+05	1.27E+06	2.83E+06	5.55E+06	10.61E+06
C.V	2.68%	0.47%	1.45%	0.35%	0.65%	1.47%
C.V Average	1.18%					
PI	0.547					

The Proportionality Index (PI) (referenced from ISO 20391-2) is used to quantify the extent to which a cell counting method adheres to the principle of proportionality. Deviations from proportionality indicate the presence of systematic or non-systematic errors in measurements, leading to a loss of measurement accuracy. A smaller PI value indicates that the instrument testing is closer to the proportionality principle, resulting in higher accuracy. As shown in the table above, the PI for the microsphere sample test is 0.547.

Linear regression analysis was performed on the microsphere test results, and the results are shown in Figure 2.

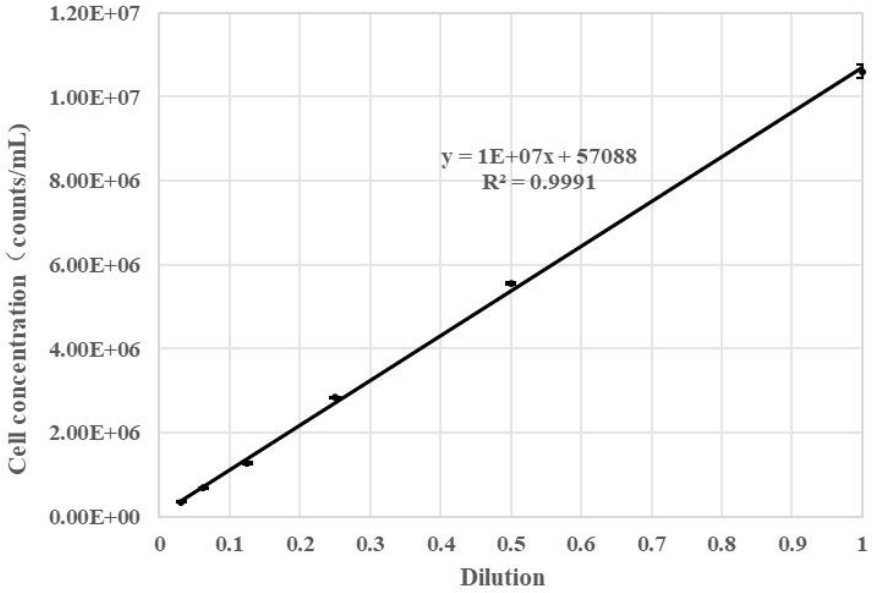

Figure 2. Linear correlation and regression analysis of the microsphere experiment

Cell Count Results

The C.V and PI for the cell sample test are shown in Table 2.

Table 2 Raw data and calculation results of cell experiment

Dilution	0.03125	0.0625	0.125	0.25	0.5	1
	2.85E+05	6.08E+05	1.29E+06	2.56E+06	4.97E+06	10.26E+06
	2.89E+05	5.94E+05	1.26E+06	2.60E+06	5.10E+06	10.16E+06
	2.80E+05	6.19E+05	1.24E+06	2.60E+06	5.17E+06	10.18E+06
TCD Average	2.85E+05	6.07E+05	1.27E+06	2.59E+06	5.08E+06	10.20E+06
C.V	1.67%	2.02%	1.89%	0.99%	1.99%	0.53%
C.V Average	1.52%					
PI	0.201					

The cell test results were subjected to linear regression analysis, and the results are shown in Figure 3.

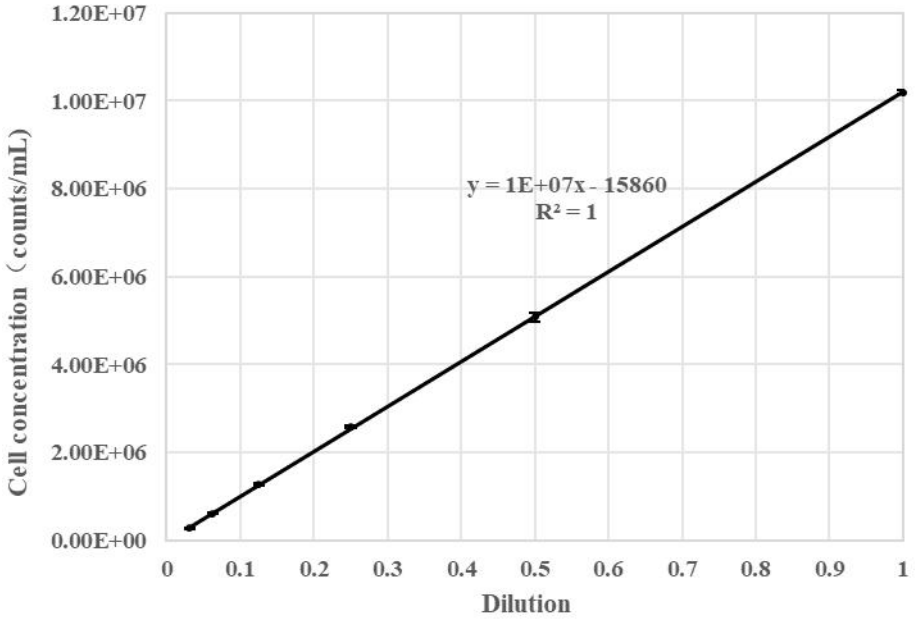

Figure 3. The linear correlation and regression analysis graph for cell experiments

Statistical calculations were performed on the viability rates for each dilution concentration, and the results are shown in Figure 4.

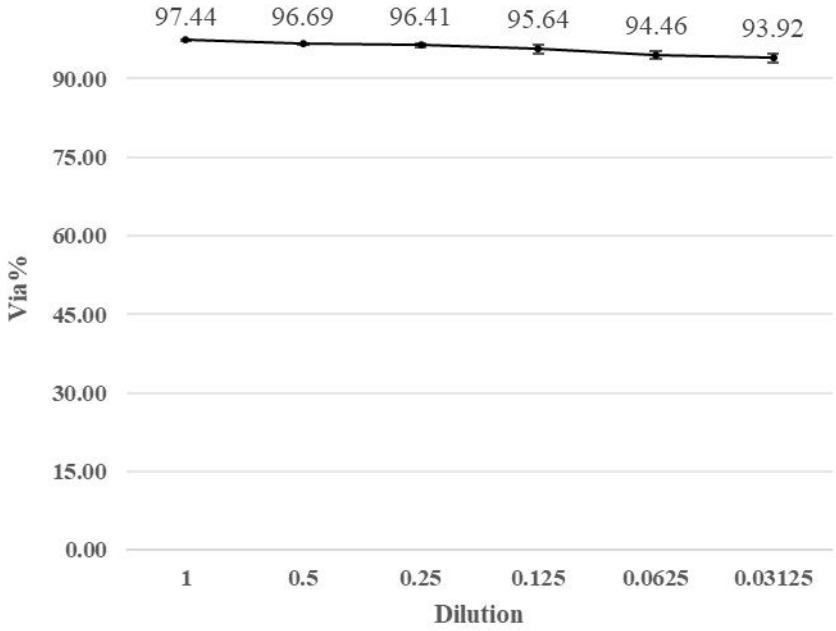

Figure 4. Viability changes at various dilution concentrations in cell experiments

Conclusion

Different experimental samples were tested on the CytScop® Pro for cell counting performance analysis. By conducting serial dilution experiments, it was observed that within the concentration range of 0.2M-10M, the CytScop® Pro demonstrates good repeatability in detecting parallel samples. The average coefficient of variation (C.V) for the microsphere experiment at each dilution concentration was 1.18%, while for the cell experiment, it was 1.52%. The C.V between parallel samples at each dilution concentration was less than 3%. Additionally, through the PI analysis of the linear experiments, it was found that the PI for the microsphere sample and cell sample were 0.547 and 0.201, respectively, indicating that the CytScop ® Pro performs cell counting with high precision.

Through linear regression analysis, it can be observed that both the microsphere sample and the cell sample exhibit excellent linear relationships in the serial dilution experiments ($R^2 > 0.999$). In fact, the R^2 for the cell samples is 1, further indicating the consistency of the counting results within the dilution range. Additionally, the cell samples have a smaller PI, suggesting that the CytScop® Pro provides cell counting results that adhere more closely to the proportional principle for cell samples, demonstrating better adaptability in cell sample testing.

An analysis of the viability of cell samples at various dilution concentrations revealed that within the concentration range of 0.2M-10M, the viability changes minimally across different dilution concentrations, with variations not exceeding 3% compared to the initial stock solution. Even at low concentrations (0.285M), the viability change is only 3.6%. This indicates that the instrument has minimal impact on the viability of normal cell samples during testing. While viability changes are also influenced by the state of the cell samples themselves, in general, for actively growing cell samples, determining concentration and viability after dilution at high concentrations and viabilities ensures that the impact of CytScop® Pro on viability is negligible and can be disregarded.

In conclusion, the CytScop® Pro intelligent cell analyzer exhibits high accuracy and precision in cell counting within the range of 0.2M-10M, showcasing the precise cell counting performance of the CytScop® Pro.

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