Fast and reliable cell counting using the Nucleocounter NC-200™

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Introduction

Precise cell counting is critical for reproducibility in cellular assays. At Aptuit (Switzerland) AG, we are cultivating a variety of different cell lines which are used for assay development and high-throughput screening for hit identification in drug discovery projects. In that regard, reproducible cell counts are crucial to obtain comparable data across different batches and days in longer screening campaigns. In addition to cell number, assessment of cell viability is particularly important for functional assays with TR-FRET and FLIPR readouts performed at Aptuit, and we highly rely on the NucleoCounter® NC-200™ (Chemometec) for robust and reliable cell counting at moderate cost with little hands on time.

Materials and Methods

Adherent, target-expressing Chinese Hamster Ovary (CHO), 1321N1 human brain (astrocytoma) and wild-type Prostate Cancer (PC3), as well as suspension THP-1 monocytic cells were grown in predefined media at 37 °C and 5% CO_2. For cell counting, adherent cells were trypsinized and re-suspended in full growth medium, suspension cells were counted without any further treatment. Via1-Cassettes™ were loaded with sample by the simple procedure of inserting the Via1-Cassettes™ tip into the cell suspension and pressing down the piston, which requires as little as 100 µl of cell suspension for cell counting.
Using the NucleoCounter® NC-200™ (Chemometec) and the standard protocol “Viability and Cell Count”, cell count and viability were determined. Furthermore, cell diameter and percentage of cell aggregates were also reported in the same determination. All measurements were performed in triplicates from the same cell suspension.

Results

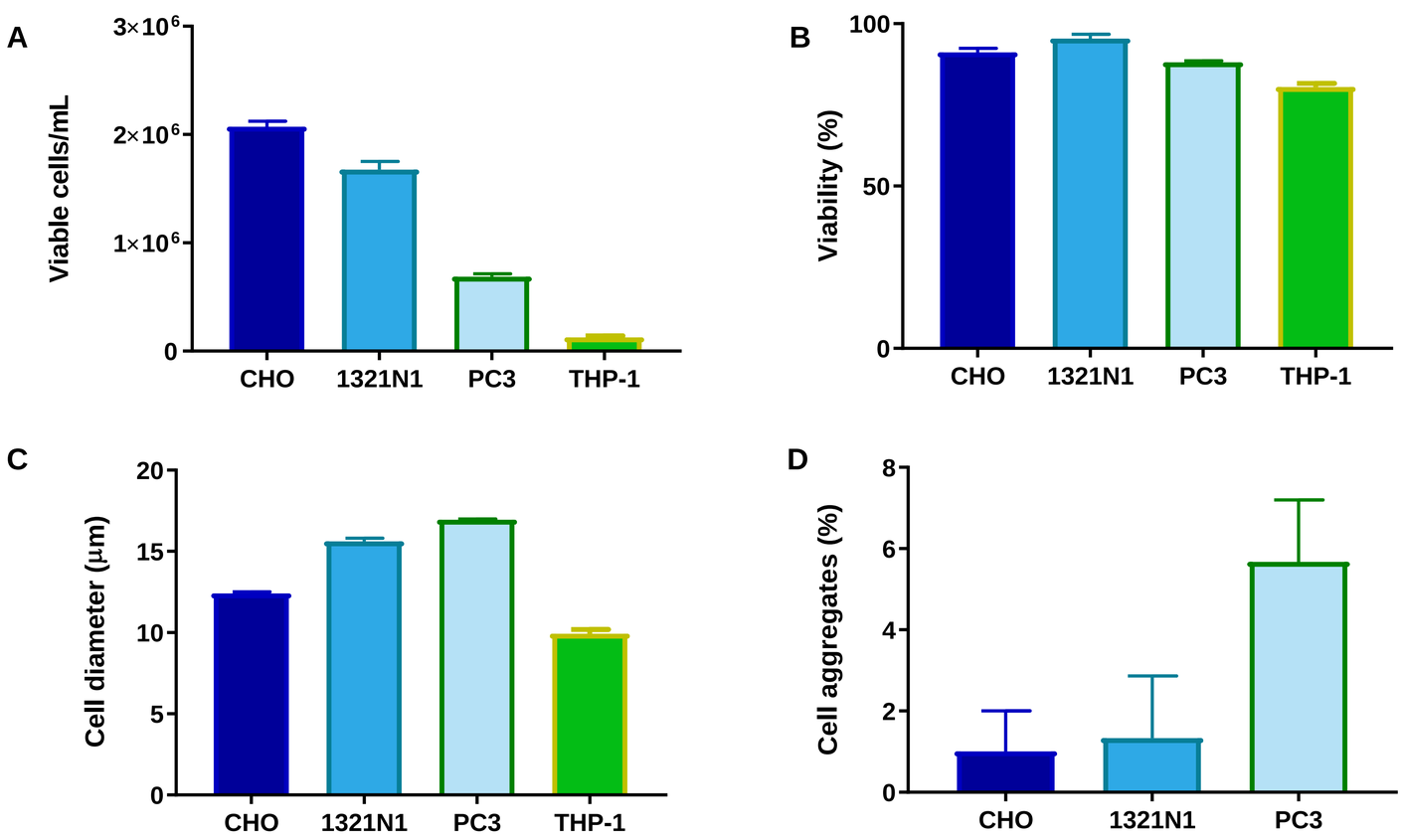

Figure 1: Cell count parameters of the adherent cell lines CHO, 1321N1 and PC3 and the suspension cell line THP-1. Cell monolayers of adherent cells were detached using trypsin, re-suspended in full growth medium and suspensions measured immediately. Suspension cells THP-1 were not further treated before measurement. All cell suspensions were loaded into a Via1-Cassette™ and analyzed on the NC-200™ for count of viable cells (A), percent viability (B), cell diameter (C) and percent of aggregates (D). No cell aggregates were detected in the suspension cell line THP-1. Data in all graphs is the average of three measurements; error bars represent standard deviation from the mean. The percent coefficient of variation (CV) of the replicates for live cell count and viability is displayed in Table 1.

Cell line	Viable cells (cells/mL)	Viable cells (CV%)
CHO	2.07 x10^6	2.6
1321N1	1.68 x10^6	4.5
PC3	0.687 x10^6	3.8
THP-1	0.127 x10^6	10.1

Table 1: Counts of viable cells (as displayed in 1A). Mean of three measurements and standard deviation in % (CV).

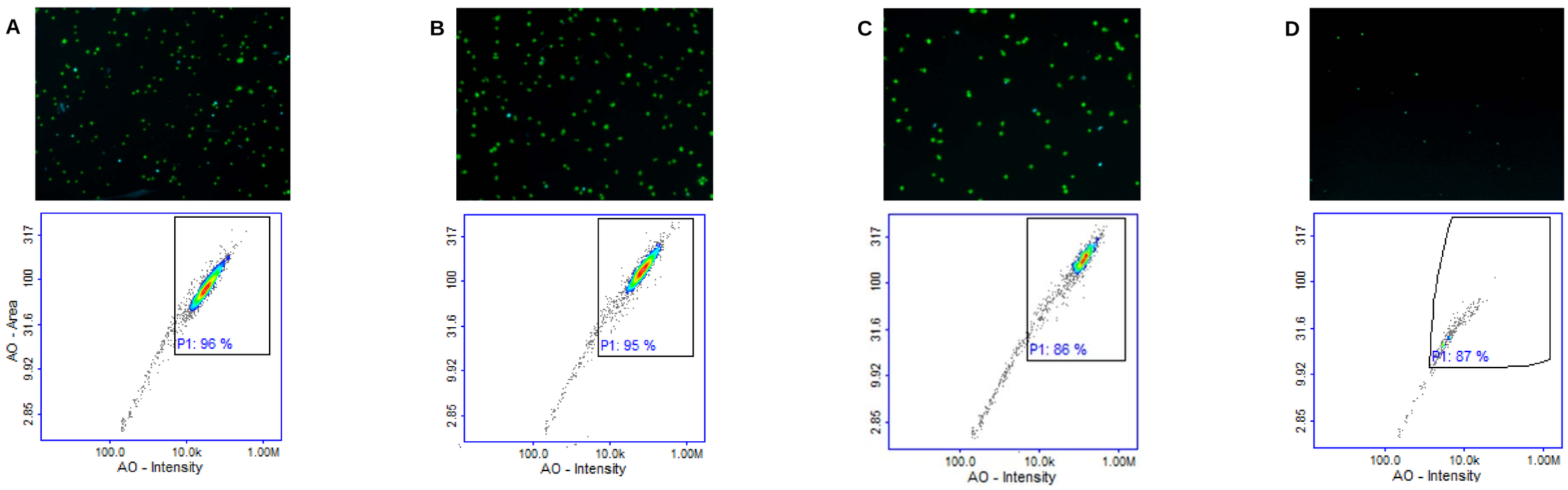

Figure 2: Representative fluorescence images of the cell lines CHO (A), 1321N1 (B), PC3 (C) and THP-1 (D) as well as associated 2D-density plots. Using the Via1-Cassette™ cells were stained with acridine orange (AO; all cells) and DAPI (dead cells). The analyzed cells displayed various morphology and clumping in the fluorescence images. The software within the instrument's software displays the raw data as representative 2D-density plots of AO staining, with fluorescence intensity (x-axis) vs. area (y-axis), P1 depicts cell viability. For THP-1 cells (E), the standard counting gate was slightly adapted and a new protocol was created.

Summary

Counting cells using the NucleoCounter® NC-200™ is a fast and reliable method to determine cell numbers and viability with low running cost and little maintenance effort. Reproducible cell counts with a low CV were obtained for all cell suspensions covering a wide range of cell concentrations and with fast throughput (<1 minute per measurement). No additives other than the Via1-Cassette™ were used for counting. The standard protocol “Viability and Cell Count” was used for the different adherent and suspension cells, even for the slightly clumpy PC3 cells. As cell aggregation never exceeded 20% there was no need to switch to the “Aggregated Cells Assay” protocol. For THP-1 cells, a new protocol was defined by enlarging the counting gate to ensure all cells were counted correctly.