fluidlab R-300

Accurate and reliable cell counting

using holographic microscopy and machine learning algorithms

Introduction

Reliable and accurate cell counts are crucial for many different purposes such as the maintenance of cell cultures in biological research or in-process controls in industrial bioprocessing. Some critical indicators of the quality of the cell culture are cell density, morphology, and viability.

When considering currently available cell counting options, many users ask which cell counting method is better: manual counting or using automated cell counters. Traditional manual cell counting involves the use of haemocytometers and requires experienced lab personnel to analyze cell samples. In addition to the inherent user-to-user variability, manual counting is also more labour-intensive than automated cell counting.

The fluidlab R-300 offers fast and accurate cell measurements by combining state-of-the-art digital holographic microscopy and fully automated analysis based on deep neural networks. The main difference between conventional automated cell counters based on brightfield microscopy and the fluidlab R-300 is that holographic microscopy provides more information about the sample and does not require any additional staining to enhance the contrast of faint biological samples. Moreover, it quantifies not only cell density, but assesses cell viability completely label-free[1].

In this study, we evaluated the performance of the fluidlab R-300 cell counter according to the recommendations of the CLSI EP05-A3[2]. To this end, we assessed accuracy, precision, linearity, and repeatability when counting three different cell types (HeLa, PBMCs and yeast) with distinct morphologies and sizes. Our data demonstrates that the fluidlab R-300 cell counter is accurate, precise and ensures linearity of the results over a large range of cell types and concentrations.

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[1] Application Note – Holographic analysis of single cells: native and staining-free pharmacologic assessment of drug dose responses (www.anvajo.com/products/fluidlab-r300)
[2] Clinical & Laboratory Standards Institute: Evaluation of Precision of Quantitative Measurement Procedures, Approved Guideline - 3rd Edition

Methods

Cell sample preparation

HeLa cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) and detached from the flask surface by trypsinization. To minimize sample variability during the cell counter validation, HeLa cells were frozen in small aliquots at a concentration of ~10^6 cells/mL in freezing medium (10% DMSO, 20% FBS in DMEM cell media) and stored at –20°C.

Similarly, yeast cells (*Saccharomyces cerevisiae*) were grown in yeast culture medium (NaCl Peptone Buffer and Saccharose) for 2 days at 20°C and then frozen at -20°C in small aliquots at a concentration of ~10^7 cells/mL in 15% glycerol (v/v) in water.

Prior to every test, aliquots of HeLa and yeast cells were quickly thawed, washed with PBS, and centrifuged. The supernatant was discarded and the cell pellet was resuspended in small volumes of HeLa or yeast cell culture medium yielding a stock concentration of ~10^7 cells/mL for HeLa and ~10^8 cells/mL for yeast cells. Nominal cell concentrations were prepared by counting the cell stock manually in a haemocytometer and preparing the respective dilutions with cell culture medium.

The human PBMC samples used in the validation experiments were isolated from buffy coats of healthy donors by Ficoll-Paque density centrifugation to ensure removal of red blood cells (RBCs) from the sample. Cell pellets were resuspended in small volumes of PBS to yield a concentration of ~10^7 cells/mL. To ensure cell viability, samples were stored at 4°C until testing on the same day (< 6 hours after isolation).

Automated cell counting

The fluidlab R-300 and a competitor brightfield cell counter were used for automated cell counting. Briefly, for measurements with the fluidlab R-300, 20 µl of cell suspension were loaded into the acella100 sample carrier, which was then inserted into the fluidlab R-300. The fluidlab R-300 counts cells in a user-defined cell size range based on digital holographic microscopy and deep neural network cell detection algorithms. Setting an appropriate size range for the cells of interest can exclude debris or other cell populations in a sample from analysis. The size range can be dynamically adjusted using the cell size histogram once cells have been counted. The anvajo datalab software was then used to export data from the device to a PC for further data analysis.

For comparative studies, counts were also performed using a competitor cell counter according to manufacturer's instructions, using the same cell samples and cell size ranges.

Results

To highlight the versatility of the fluidlab R-300 for automated cell counting, we tested the performance of the fluidlab R-300 on three different cell types that exhibit distinct morphologies and sizes (Figure 1). **HeLa cells** are the first-ever immortal human cell line which is still commonly used in biological and clinical research. HeLa cells are normally 20 – 40 µm in diameter depending on the culture conditions. In contrast, **peripheral blood mononuclear cells (PBMCs)** are among the smallest human cells with a diameter of around 7 – 10 µm. PBMCs are a common primary cell source used in a wide range of research studies in clinical diagnostics and immunology. It can be challenging to accurately quantify the cell concentration of primary cell samples using traditional cell counting methods, because they often contain a heterogeneous collection of cell types and cellular debris. Finally, we used **yeast cells** (*Saccharomyces cerevisiae*) with an average size of approximately 5 µm to assess the cell counter performance on very small cells. Yeast is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, but it is also critical to the fermentation process in beer brewing and wine industries.

HeLa cells	PBMCs	Yeast
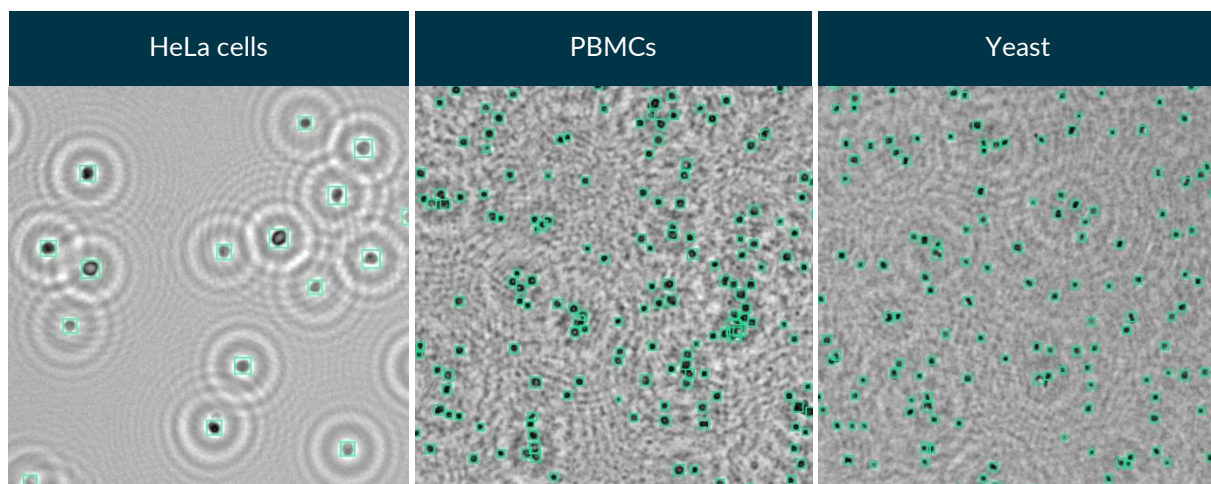
Fig1.: Counting of HeLa cells (left), freshly isolated peripheral blood mononuclear cells (PBMCs, middle) and yeast cells (right) with the fluidlab R-300. The holographic microscopy image allows researchers to verify the cell count results based on the green boxes highlighting detected cells. Moreover, cell morphology and the degree of homogeneity of the sample and the presence of debris or dirt in the sample can be evaluated. For better visibility, only a small image section and not the whole field of view (5.3 mm^2) is shown here.

The fluidlab R-300 cell counter covers a large concentration range

The fluidlab R-300 can automatically count cells over a large range of concentrations (10^4 - 10^7 cells/mL). To assess linearity in comparison to a competitor cell counter, serial sample dilutions of three different cell types (HeLa, PBMCs, yeast) were tested over the specified concentration range. Figure 2 shows the cell count for the three different cell lines obtained with the fluidlab R-300 in comparison to the competitor device. The high degree of linearity (as shown by the R^2 values of the linear regression) shows that the fluidlab R-300 cell counter reliably detects and counts different cell types across a wide linear operating range. Moreover, the overall total count measured with the fluidlab R-300 is comparable to the total count of the competitor device (as indicated by the slope of the linear fit being close to 1).
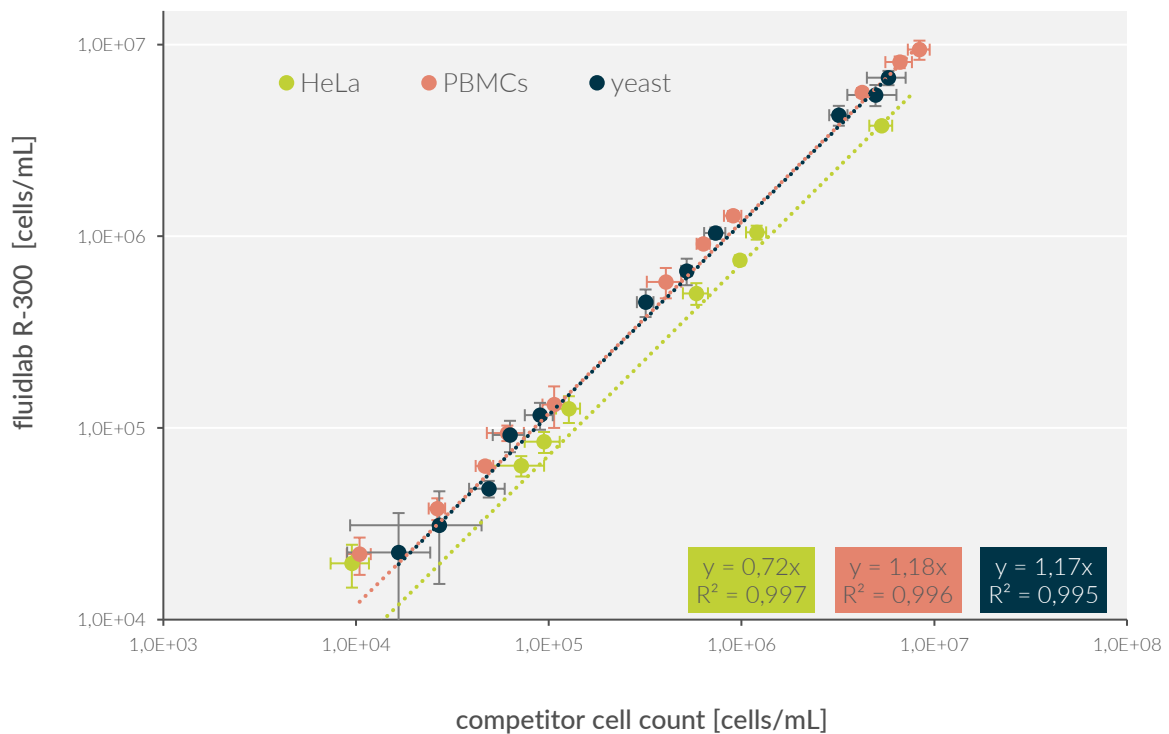
Fig2.: The fluidlab R-300 performs with high linearity across different cell types over a large concentration range. To assess linearity of the fluidlab R-300 in comparison to a competitor cell counter, different concentrations of HeLa cells, PBMCs and yeast were measured in triplicate and on three different days. All measurement results for a given nominal concentration were averaged over all replicates (N=9) and the standard deviation was calculated to assess variability. The same procedure was performed using the competitor cell counter. Error bars denote standard deviations.

Accurate and precise cell counting with the fluidlab R-300

The accuracy (also known as trueness) of a cell counter describes how close the measured value is to a 'standard' or known value, while precision refers to the closeness of replicate measurements to each other. For cell counting, the manual microscopic count using a haemocytometer is often regarded as the standard reference method. However, manual counting is also error-prone, time-consuming, and highly dependent on the level of user expertise.

Here, we compared the cell count obtained with manual counting, the fluidlab R-300 and a competitor cell counter for three different cell types and concentrations. All the data obtained are summarized in Figure 3. The accuracy of the fluidlab R-300 cell count is comparable to manual counting within a concentration range of 10^5 to 10^7 cells/mL. At lower concentrations, the accuracy differs from manual counting but is comparable to the competitor cell counter. This is likely associated to poor statistics when less cells are detected in the field of view. As cell concentrations increased the error decreased, because a larger number of cells are included in the analysis. For the majority of tested cell types and concentrations, the fluidlab R-300 cell count is highly precise (as indicated by the small error bars in Figure 3). Overall, the fluidlab R-300 provides precise and accurate cell counting results over a large range of cell types and concentrations – in less than 30 seconds per sample.
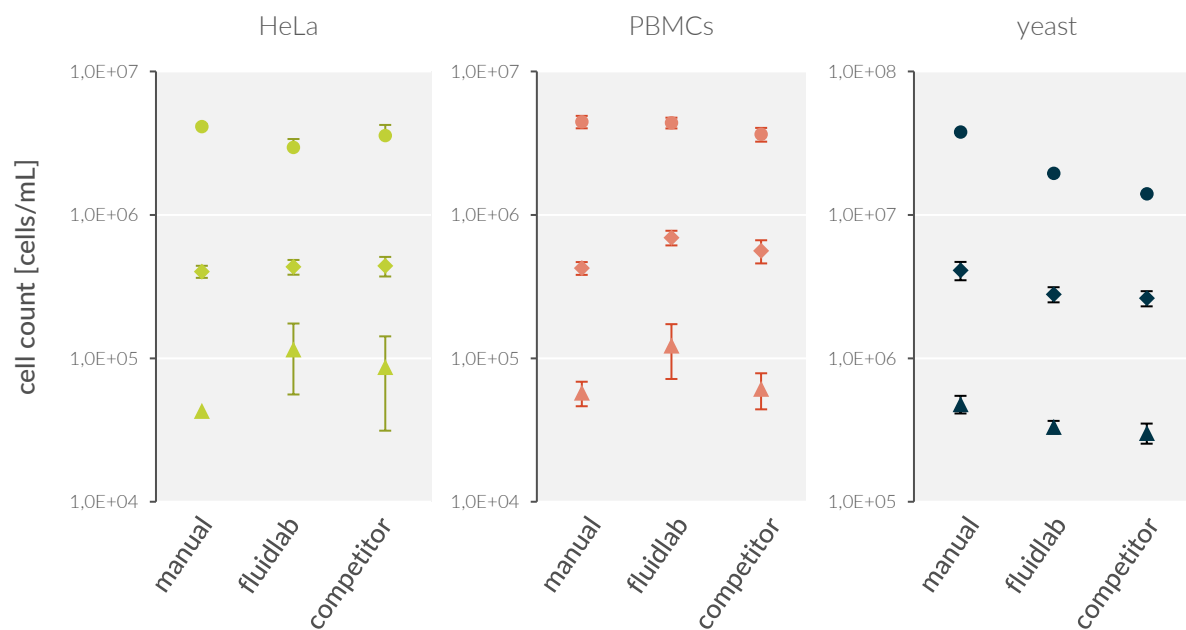
Fig3.: To determine the accuracy and precision of the fluidlab R-300 cell counter, every sample was counted three times manually in a haemocytometer to determine the standard value and then counted 10 times using the automated cell counter. We performed this test for 3 different cell lines at 3 different concentrations for both the fluidlab R-300 and a competitor cell counter. All measurement results for a given concentration were averaged across all replicates (N=10) and standard deviations were calculated to assess variability. Error bars denote standard deviations.

Automated cell counting ensures reproducible results

To ensure reproducibility in experiments and manufacturing processes, it is essential that automated cell counting is highly repeatable with minor variability from count to count.
To assess repeatability of the fluidlab R-300 cell counter, we tested three different cell types (HeLa, PBMCs, yeast) at three different concentrations on 10 separate days. To minimize potential variability in the experimental conditions, frozen stocks for yeast and HeLa cells were used, while PBMCs were freshly isolated from blood every day.

Figure 4 shows the measurement results for the three tested cell lines and concentrations for both the fluidlab R-300 and the competitor cell counter on 10 different days. The day-to-day variability likely reflects differences in experimental conditions and is consistent for both cell counting methods. Table 1 reports the inter-assay repeatability across different days and different times of the day for all tested cell lines and concentrations. Overall, the inter-assay coefficient of variation (CV) is smaller for the fluidlab R-300 than for the competitor cell counter – highlighting that the total cell count obtained with the fluidlab R-300 is very reproducible across different days.
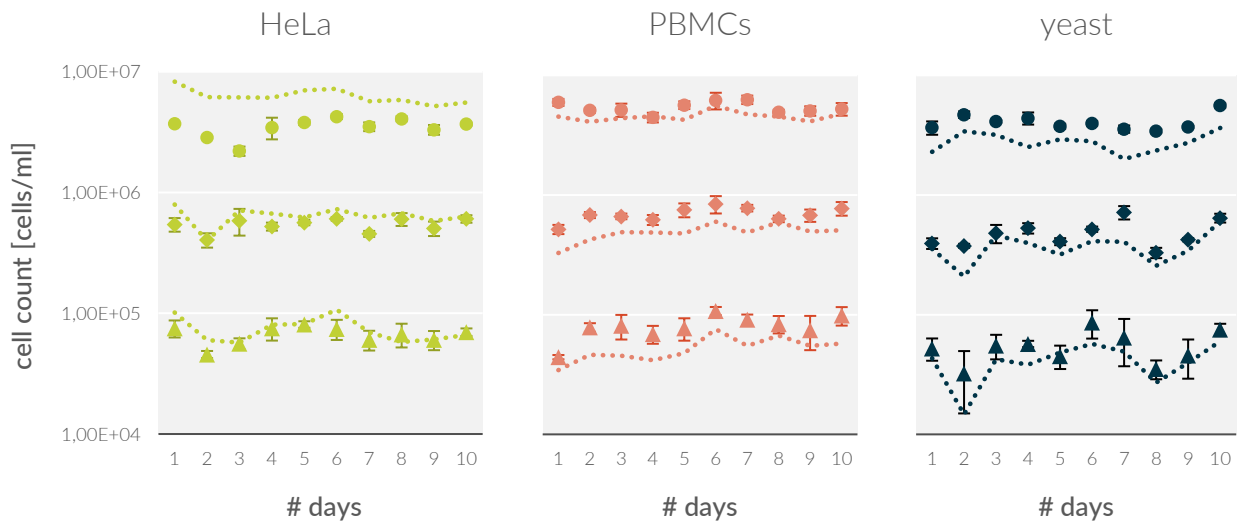
Fig4.: To validate repeatability of the fluidlab R-300 cell counter, multiple samples were measured on 10 days, with two runs per day (morning and afternoon), and two replicates per run. For each cell line (HeLa, PBMCs and yeast), three distinct concentrations were tested. Solid symbols denote averages of replicates on the same day (N=4) for the fluidlab R-300. Error bars show standard deviations. The competitor cell counter results are shown as dotted lines.

HeLa

nominal concentration [cells/mL]	fluidlab R-300		competitor	
	mean [cells/mL]	CV	mean [cells/mL]	CV
5,00E+04	6,64E+04	16%	7,42E+04	24%
5,00E+05	5,39E+05	13%	6,43E+05	17%
5,00E+06	3,76E+06	10%	6,32E+06	15%

PBMCs

nominal concentration [cells/mL]	fluidlab R-300		competitor	
	mean [cells/mL]	CV	mean [cells/mL]	CV
5,00E+04	8,04E+04	21%	5,25E+04	23%
5,00E+05	7,00E+05	14%	4,89E+05	16%
5,00E+06	5,39E+06	11%	4,55E+06	9%

yeast

nominal concentration [cells/mL]	fluidlab R-300		competitor	
	mean [cells/mL]	CV	mean [cells/mL]	CV
5,00E+04	5,45E+04	31%	4,20E+04	32%
5,00E+05	4,79E+05	26%	3,76E+05	29%
5,00E+06	4,07E+06	17%	2,79E+06	19%

Table1: Summary of the inter-assay variability for three different cell types at three different nominal concentrations across 10 different days (with duplicate measurements in the morning and afternoon, N=40). Automated cell counting of each sample was performed both with the fluidlab R-300 and the competitor cell counter.
The comparably lower coefficient of variation (CV) of the fluidlab R-300 shows that cell count results are very reproducible across all tested cell types and concentrations.

Summary

Here, we showed that the fluidlab R-300 can be successfully employed for automated cell counting across a broad range of cell types, including heterogeneous samples and small cells such as PBMCs and yeast cells. Its results are accurate, precise and highly repeatable across a broad concentration range – a fundamental prerequisite for using the fluidlab R-300 in various applications like monitoring cell proliferation, cell culture maintenance and bioprocessing.
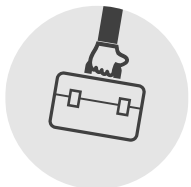
Simple – cell counting without any staining
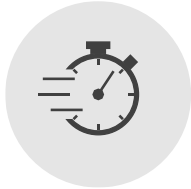
Fast – results in less than 30 s

Convenient – analysis of a wide range of cell types

Small – cell analysis directly where you need it

Intuitive – no extensive user-training required