Camilla Domeneghetti, Caitlin Davies, Stefan Borasinski, Advanced Instruments

Overview

The Viability application on the ICON instrument was investigated in its ability to out-perform an established competitor automated cell counter in the detection of sample concentration and viability. A range of viabilities and concentrations across three cell lines were used to determine inter- and intra-sample variation, results of which indicate that ICON's artificially intelligent detection is notably more accurate than its competitor.

Introduction

Cell counting and viability assessments are vital in cell culture and the cell line development process, yet the use of a haemocytometer is time consuming and highly inaccurate due to user bias.

The Trypan Blue Viability assay on the ICON instrument uses an artificially intelligent neural network to overcome user bias and reduce time spent counting with a haemocytometer or visually calibrating other automated cell counters. The assay requires just 20 µl of sample and can measure up to 24 samples at a time in only three minutes, making it ideal for both static and suspension culture deep well plates. ICON's Viability results can be uniquely combined with results of the ICON low volume titer assay and analysed by STUDIUS to depict protein productivity for selection of high value clones. Furthermore, STUDIUS can combine Cell Metric® clonality and outgrowth data with ICON viability and titer data to make rapid, unbiased and holistic clone selection decisions.

The objective of this study was to therefore validate ICON's ability to correctly identify sample concentration and viability, and out-perform a competitor automated cell counter across CHO and HEK cell lines.

Methods

Materials

- 293T adherent cell line cultured in DMEM High Glucose + 10% FBS (Gibco)
- Expi293F suspension cell line cultured in Expi Expression Medium (Gibco)
- CHO-S suspension cell line cultured in CD OptiCHO + 8mM GlutaMAX (Gibco)

Concentration (cells/mL)	Dilution Fraction	Viability (%)				
1×10^5	0.01	0	50	70	90	100
1×10^6	0.30	0	50	70	90	100
1×10^6	0.50	0	50	70	90	100
1×10^6	0.70	0	50	70	90	100
1×10^7	1.00	0	50	70	90	100

Table 1: Theoretical range of sample concentrations and viabilities to be tested for each cell line.

For each cell line, an initial 1×10^7 cells/mL sample was prepared at ~100% viability and ~0% viability respectively. These samples were then mixed proportionally and diluted with 1X PBS to produce a range of theoretical viabilities (0% to 100%) and concentrations (1×10^5 cells/mL to 1×10^7 cells/mL) for each cell line (Table 1).

Inter and Intra Sample Variation

Each sample was divided into two 20 µl samples for ICON and the competitor counter respectively. Samples for ICON were mixed with 80µl filtered 0.125% Trypan Blue (Gibco) and samples for the competitor counter were mixed with 20µl 0.4% Trypan Blue (Gibco). From these mixtures three cell counting slides channels per instrument were loaded and the viability and cell concentration of each channel was recorded three times.

Stability Across 24 Samples

Of the 5x10 cells/mL concentrations, 480µl of filtered 0.125% Gibco Trypan Blue was mixed with 120µl of each viability sample. 24 ICON Cell Counting Slide channels (three slides) were then loaded and the viability and cell concentration of each channel was recorded a single time.

Results

Concentration and viability linearity was determined and a simple linear regression was conducted (Figure 1). The inter- and intra-channel coefficient of variation (%CV) for each of the three repeats of the three replicate channels for every concentration, viability and cell line was calculated (Figure 2).
Mean Average Precision (mAP) from preliminary network validation (Figure 3) and visual analysis of exported images (Figure 4) were used to further assess ICON's accuracy and precision of cell detection.
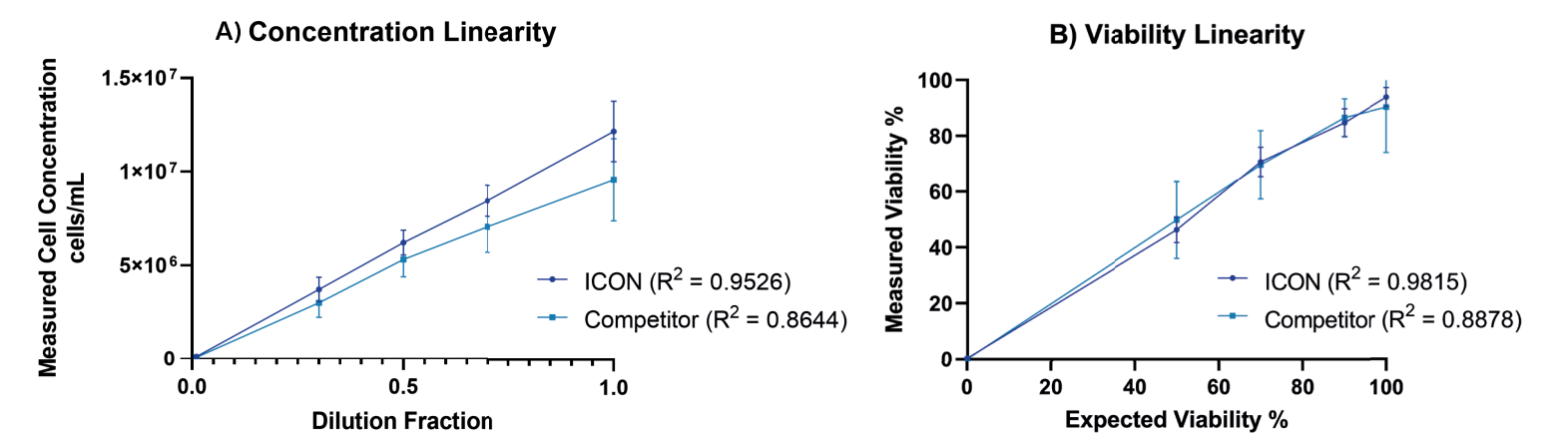

Figure 1: Linearity of A) concentration and B) viability measurements across CHO-S, Expi293F and 293T cell lines (n= 135 ± standard deviation).
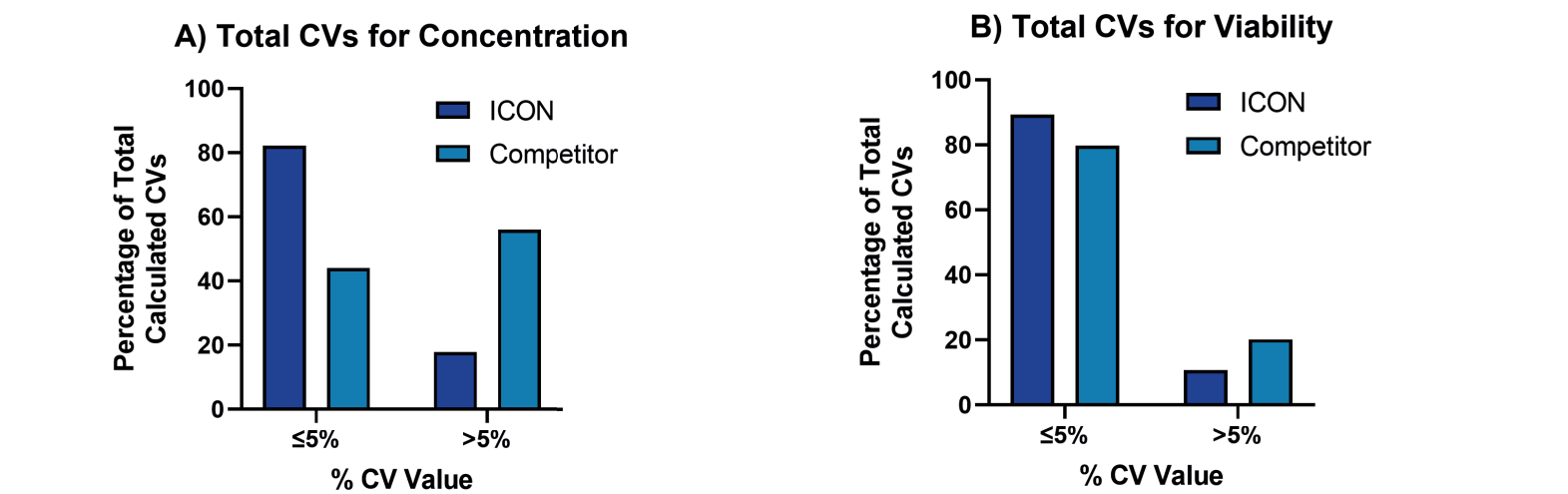

Figure 2: Percentage of total inter- and intra-channel %CVs that show a precision of ≤5% across all cell lines for A) concentration and B) viability (n=450).
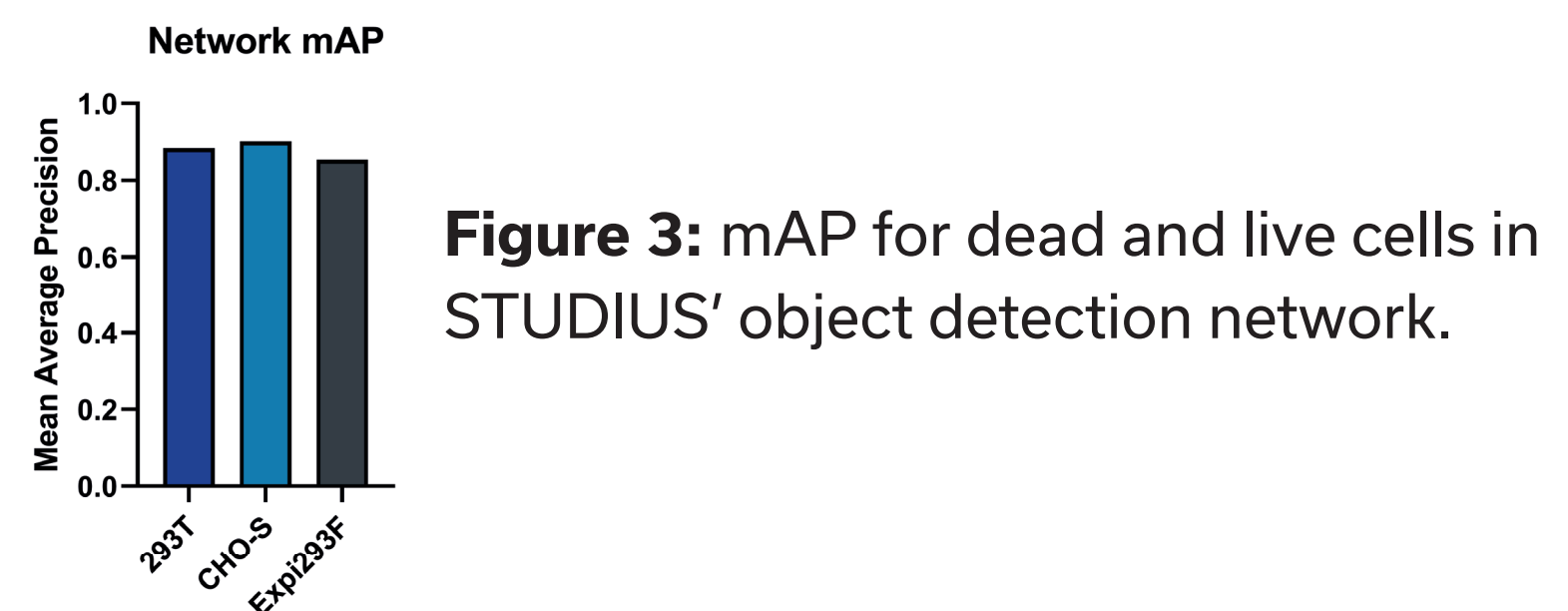

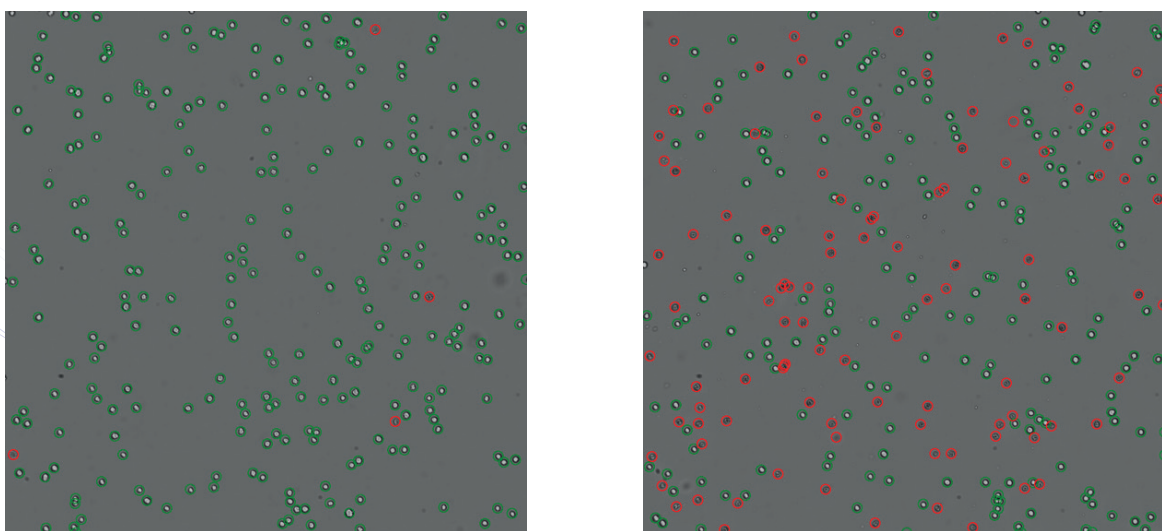**Figure 3:** mAP for dead and live cells in STUDIUS' object detection network.

Figure 4: Network detection of CHO-S cells at approximately 5x106 cells/mL with an expected viability of 90-100% (left) and 70% (right).

Conclusion

- The linear regression analysis shows high precision and low variability in ICON, with R^2 values of 0.95 and 0.98 for concentration and viability respectively which is evidently higher that the competitor values of 0.86 and 0.89.
- ICON displays highly consistent and reliable measurements with 82% of 450 triplicate repeats having a coefficient of variation ≤5% for concentration and 89% for viability. This increases to 93% for both concentration and viability when using a precision threshold of CVs being ≤10%. This again is significantly higher than the competitor instrument that has only 44% and 80% of CV's ≤5% for concentration and viability respectively.
- The mAP scores range between 0.85 and 0.90, confirming the networks strong ability to evaluate viability. Visual analysis of images from ICON again confirm the network is highly accurate at detecting and distinguishing between live and dead cells.

The ICON easily out-performs the competitor cell counter and is extremely competent at identifying cell concentration and viability. The ability to use low sample volumes of 20µl, run up to 24 samples at once with minimal requirement for user calibration or gating, allows cell line development workflows to be streamlined and accelerated. The ability to track and manage the viable concentration data in STUDIUS allows rapid assessment of samples with high confidence levels.