Real-time monitoring and comparative analysis for density and viability of CHO-M cell in SB10-X shaking bioreactor using the C-Netics

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Abstract

The C-Netics monitored cultured Chinese Hamster Ovary (CHO) cells in a Kühner SB10-X bioreactor with a 3L adapter over a 19-day period. Seamlessly connected to the single-use bioreactor bag, the C-Netics provided continuous, real-time measurements of cell density and viability throughout the entire run. Comparative analysis with gold standard laboratory devices revealed results comparable with those analyzers commonly used in biomanufacturing process. This study underscores the C-Netics' compatibility with shaking bioreactors and its capacity to deliver dependable, real-time monitoring, positioning it as a pivotal tool for advancing bioprocess control and optimization.

Introduction

Background: Accurate cell count and viability assessment are crucial for monitoring cell cultures. Traditionally, off-line analysis involving manual sampling from bioreactor contents is standard practice. Manual cell counting using dye exclusion methods is cost-effective but may overlook early-stage dying cells and is time-intensive. Detecting apoptotic cells early poses challenges; however, leveraging the electrical properties of cells can detect early changes in ionic composition and dielectric properties. Existing devices based on this principle have inherent limitations, yet cell electrical parameters are already utilized for counting and viability assessment. Flow cytometry offers comprehensive data but is costly and necessitates multiple preparation steps.

Novelty: Current methods for measuring cell count and viability involve manual sampling or the use of automated devices that require several manual steps, dilutions and the use of captive reagents. Manual sampling has several drawbacks, including daily handling, significant volume loss, and contamination risk. In contrast, the C-Netics presents a pioneering approach for continuous analysis of cells in suspension. This innovative device enables label-free, single-cell analysis using dielectrophoresis, microscopy, and advanced image processing techniques. It effectively detects dead cells, through online, automatic analysis without exposing the sample to external environments. The closed-loop system of the C-Netics device enables real-time monitoring and control of critical process parameters while maintaining sample sterility and integrity. With no need for calibration, its single-use kit remains effective throughout the entire culture duration, providing measurements of cell density, viability, and visual properties seamlessly.

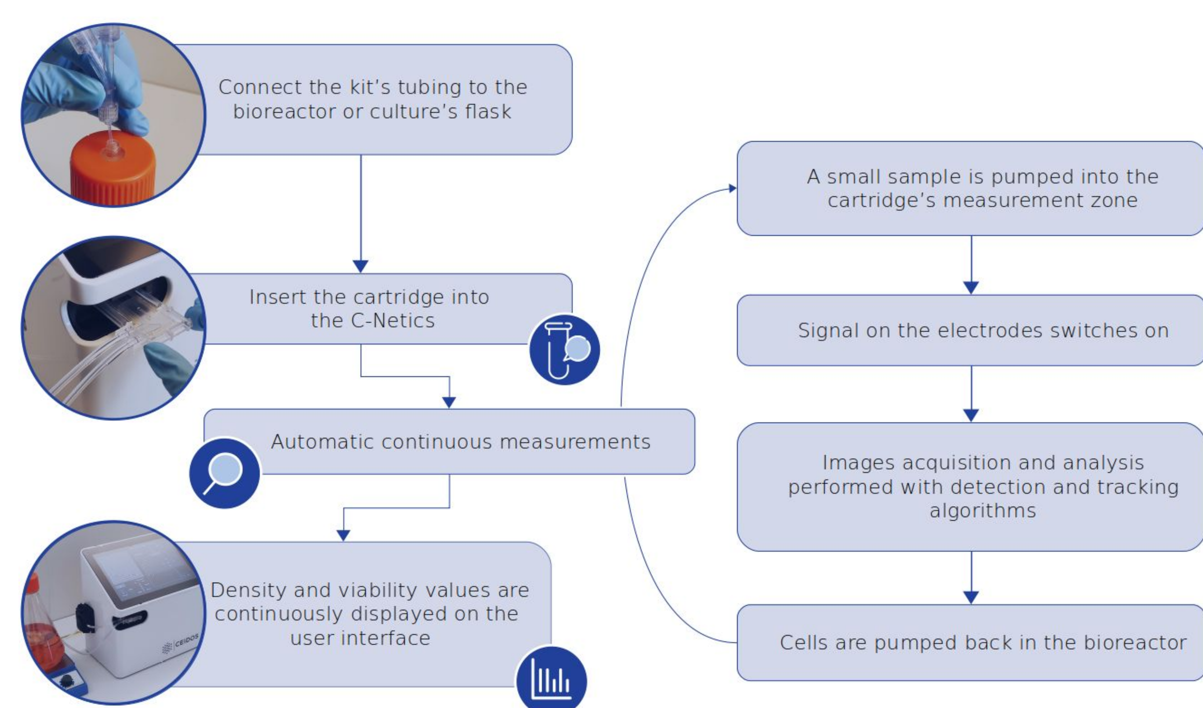

Figure 1: Diagram illustrating the operating principle of the C-Netics from connection to the cell culture to the end of the bioprocess.

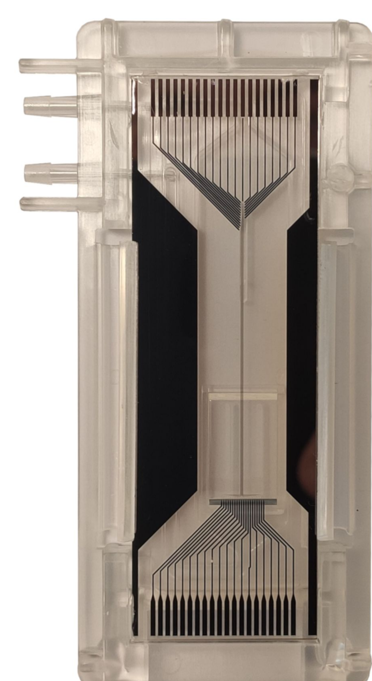

Figure 2: Microfluidic cartridge where the cells are analyzed

Material and Method

Process: CHO M cells (cell line developed in Prof Hagens lab at HES-SO) were cultivated in an Kühner SB10-X bioreactor (Kühner shaker) with a working volume of 1.5 liter. The cells were inoculated at a concentration of 0.35*10⁶ cells/ml and expanded for 19 days under specific conditions: 40% dissolved oxygen (DO), shaking speed at 90 rpm (adjusted to have a single wave) and pH 7.0. The temperature was set at 37°C at first and then a temperature shift to 32 °C was done at day 7. Cells were fed from day 4 with 1% Feed A and 0.5% Feed B.

Set-up: Single-use bag was installed in the bioreactor on the first day, and pH and DO probes were connected. Peripherals were welded on the bag and Ceidos single use kit was connected under laminar flow through a Luer-Lock connector.

C-Netics and single-use kit: The C-Netics was connected during the entire culture duration. Every 20 minutes, density measurement is performed and also allows to determine morphological properties at a single-cell level, such as diameter and roundness. Viability measurements are performed thanks to high frequency signals triggering traveling-wave dielectrophoretic forces on the cells were turned on to determine viability. Cell detection and tracking were performed during cell migration. The cartridge used for this test had an electrode width of 13 µm with 7 µm gaps in between.

Comparative analysis on density and viability measurements: On a daily basis, the sampling line is manually purged by discarding a 5 mL sample from the bioreactor using a sterile syringe. Following this, a 4 mL sample is collected using another sterile syringe. Various analyzers are then used to measure cell density and viability. These analyzers include the Vi-CELL BLU (Beckman Coulter), the NucleoCounter NC-100 (Chemometec), the Casy Counter (OMNI Life Sciences), and Cytodirect (Mettler Toledo). Additionally, manual counting is performed using a counting chamber.

Data analysis: Density is measured using 10 raw images. Detection is performed using an in-house developed algorithm currently requiring a correction factor due to non-standardized cartridge assembly; this step will be eliminated with the industrialized version. Viability measurements involve assessing the speed of cells on electrodes under dielectrophoretic forces, with a predefined speed threshold from previous experiments used to classify cells as living or dead. These results are validated against reference devices.

Figure 3: The C-Netics connected to SB10-X bioreactor. The samples are pumped from the bottom of the bag through the C-Netics single-use kit, where they are analyzed. The same line is used to pump cells back in the bag.

Results and discussion

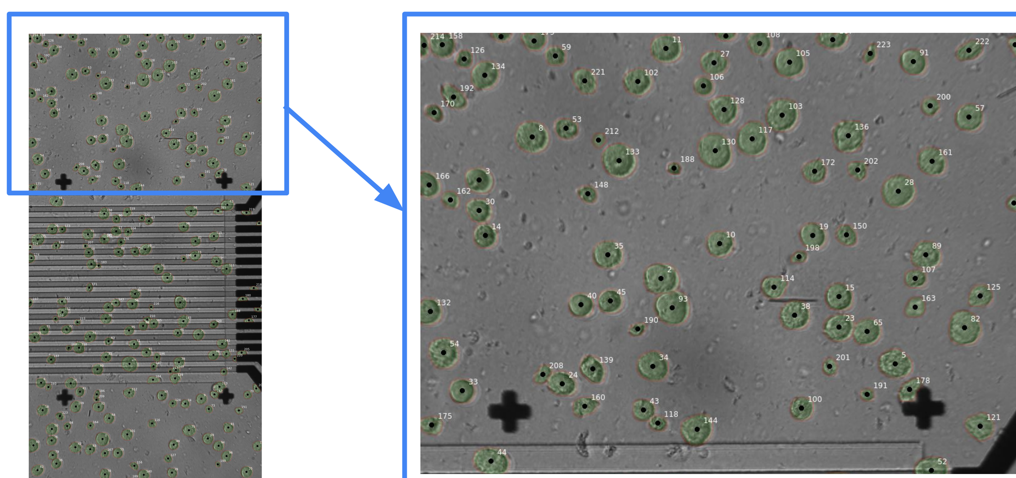

Figure 5: Image acquired with the C-Netics with cells detected highlighted in green.

Detection and tracking

Cell detection and tracking are automatically performed using previously trained models. Figure 5 shows a raw image of cells in the measurement zone, acquired with the C-Netics system, with detection overlays. In the image, individual cells are highlighted in green, with their centers represented by black dots. Despite the presence of considerable amount of debris, the algorithm effectively distinguishes cells from debris, successfully detecting only the actual cells.

Cell tracking enables the determination of the speed of each individual cell on the electrodes induced by dielectrophoresis. As viability decreases over time, cells typically become smaller and move more slowly on the electrodes.

To visually represent the two distinct populations of living and dead cells, scatter plots were generated (Figure 6), with the x-axis representing speed on the electrodes and the y-axis representing cell equivalent diameter. These scatter plots cover the entire batch and various viabilities (97%, 70%, and 40%), offering a clear view of how the distributions of these populations evolve over time.

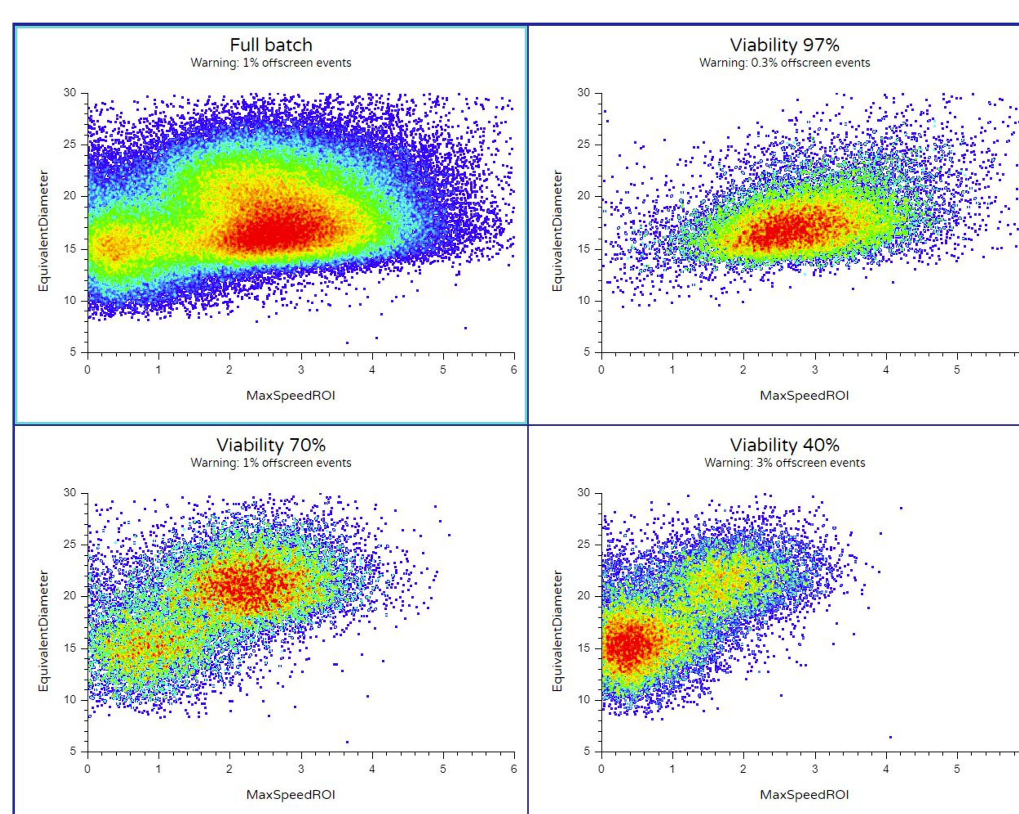

Figure 6: Scatter plot of cells at different viabilities during the run. X-axis represents speed on the electrodes and y-axis represents cell's equivalent diameter. Top left shows all cells from the batch, top right shows cell population at 97% viability, bottom left shows cell population at 70% viability and bottom right represents cell population at 40% viability

Comparative analysis on density and viability measurements

Density and viability were measured every 20 minutes, with respectively 10 images and 10 cell migrations of 15 seconds.

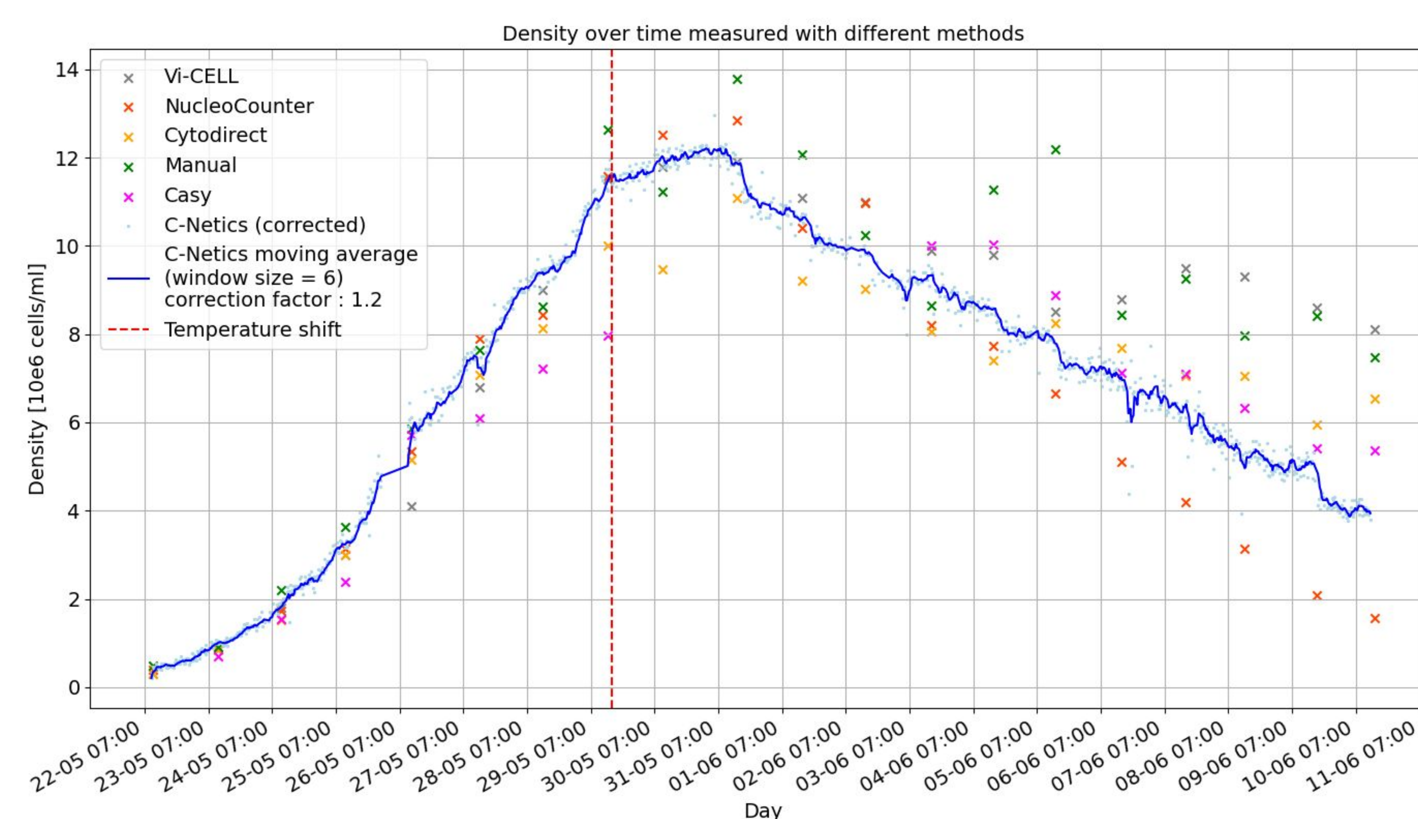

Figure 7: Total cell density measurements obtained with the C-Netics compared with values obtained with the reference measurement devices.

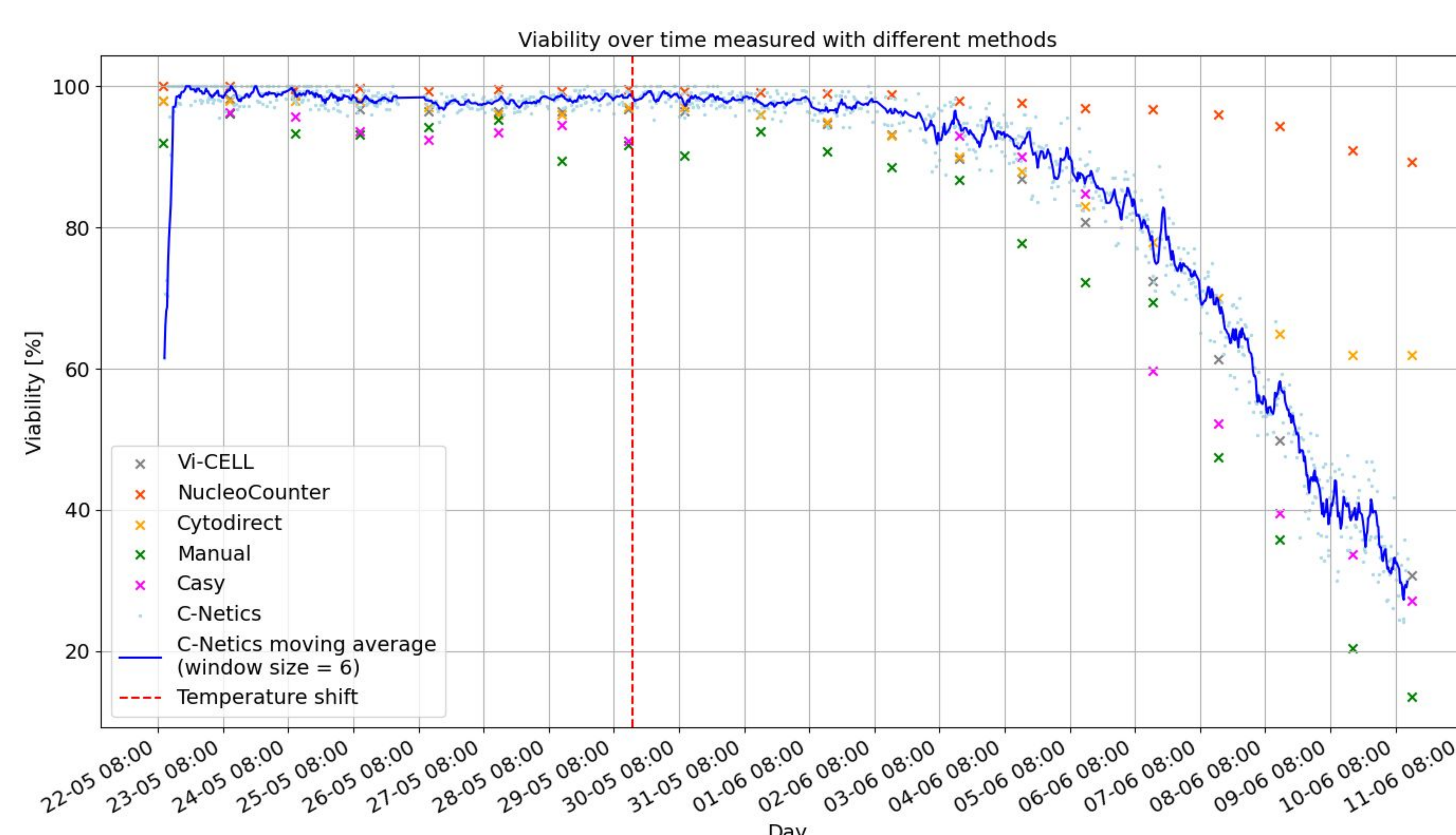

Figure 8: Cell viability measurements obtained with the C-Netics compared with values obtained with the reference measurement devices.

Over time, the C-Netics reliably follows the trend observed by the gold standard method. Significant discrepancies are noted among the other analyzers, particularly apparent at high cell densities and in the presence of debris. Toward the end of the experiment, all analyzers exceeded their measurement ranges, necessitating dilution and potentially introducing inaccuracies. The NucleoCounter showed inconsistency in the presence of debris, necessitating significant additional dilutions for accurate measurements. Additionally, the Vi-CELL mistakenly identified debris as cells, resulting in inflated cell density measurements. The Cytodirect required multiple measurements due to inconsistent results, with only corrected data points included in the analysis. Furthermore, the Casy Counter required recalibration to adjust for variations in cell size, impacting the comparability of its results. Despite these challenges encountered by the other analyzers, the C-Netics provides results by monitoring the cells online, consistently differentiating cells from debris and delivering robust and dependable results, underscoring its reliability in complex analytical environments.

Conclusion

In conclusion, the connection to the SB10-X is quick, safe and convenient and allows the C-Netics to deliver density and viability measurements comparable with gold standard methods, offering the crucial advantage of eliminating the need for sampling by providing in-line measurements. Unlike other analyzers that contend with challenges such as dilution requirement, detection errors, and calibration issues, the C-Netics excels in effectively distinguishing cells from debris and consistently providing precise results. This underscores the C-Netics as a valuable tool for continuous and accurate monitoring in bioprocessing environments, providing real-time data collection and analysis to enhance production efficiency and ensure rigorous quality control.

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