Application Note

IPRASENSE
CELL CULTURE MONITORING INSTRUMENTS

Keywords or phrases

Bioreactor, cell density, CHO, viability, seed perfusion, fed-batch production

Viable Cell Density Monitoring in Bioreactor

Abstract

Analyzing cell density and viability in mammalian cell culture bioreactors is essential, but there can be complexities. Traditional methods involve frequent sampling and staining, which is particularly problematic in small-scale bioreactors. **NORMA** analyzers offer a revolutionary solution, providing accurate measurements of cell concentration and viability without the need for staining or sample preparation. Comparative studies demonstrate the close correlation between **NORMA** XS and established methods. The advantages of this technology, including minimal sample volume, label-free detection and high repeatability, make it an ideal candidate for small-scale bioreactors and high-throughput applications. **NORMA**'s potential impact extends from cell line engineering to large-scale manufacturing, promising to revolutionize VCD measurement and process control.

Introduction

Monitoring cell density and viability of mammalian cell culture bioreactors is a necessary task that present today a number of remaining challenges. Traditional measure for cell count and cell viability still relies on sampling and staining protocol where the trypan blue exclusion method is performed once a day. While automatic cell counters have reduced the statistical error of the original manual method, daily sampling is still a challenge for small scale bioreactor because the sampled volume becomes significant.

NORMA analyzers integrate a new breakthrough method for accurately determination of cell concentration and viability without staining nor sample preparation or dilution. Two comparative studies between our **NORMA** XS and one of the reference methods showed the close correlation between our device and the reference counters. A first one concerning the **NORMA** XS and the Beckman Coulter Vi-Cell XR on the perfusion and fed-batch processes; and a second one comparing in parallel the **NORMA** XS, the Vi-Cell XR, and the Nova BioProfile FLEX on 12 fed-batch bioreactors. Moreover, we assess the very high reproducibility of such technique with a low sample volume of 3µL.

Material & Methods

The **NORMA** XS is very easy to use: the sample is simply placed on the measuring area of the slide without dilution or labelling. Then, the technology directly acquires the light diffraction properties of each individual cells through their hologram images without any settings. Several thousands of holograms are acquired into one single image that is immediately "reconstructed" with holographic algorithm to obtain a microscopic like picture; at this stage cells can be segmented. Living and dead cells have significant holographic patterns that can be distinguished and accurately counted.

A.

B.

Several thousands of cells counted within a single image gives unmatched rapidity and repeatability.

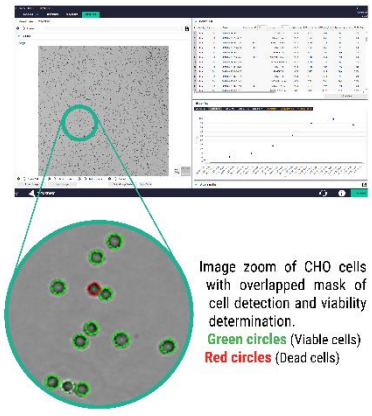


Image zoom of CHO cells with overlapped mask of cell detection and viability determination.
Green circles (Viable cells)
Red circles (Dead cells)

Figure 1: ***NORMA*** *XS with its HORUS software. A)* ***NORMA*** *XS and the deposit stage in the slide. B) The HORUS software used to control and analyze the* ***NORMA*** *XS.*

Three case studies were conducted to demonstrate the strong correlation between the new **NORMA** technology and the reference methods Vi-Cell XR and BioProfile FLEX, as well as the very good repeatability of measurements performed with the **NORMA** XS.

1. **A strong correlation between the NORMA XS and the Beckman Coulter Vi-Cell XR on the perfusion and fed-batch processes.**

A first comparative study between the Beckman Coulter Vi-Cell XR and our **NORMA** XS was realised on CHO cells on two different processes: the perfusion and fed-batch mode. A measurement was performed every day on the **NORMA** XS and Vi-Cell XR, except weekends for the fed-batch run (day 2, 3, 9 and 10 on the graph in **Figure 2**). The results showed that **NORMA** XS trended close to the reference method Vi-Cell XR for VCC (Viable Cell Count) up to 40x10^6 cells/mL and viability from 0 to 100% for both perfusion and fed-batch processes (**Figure 2**).

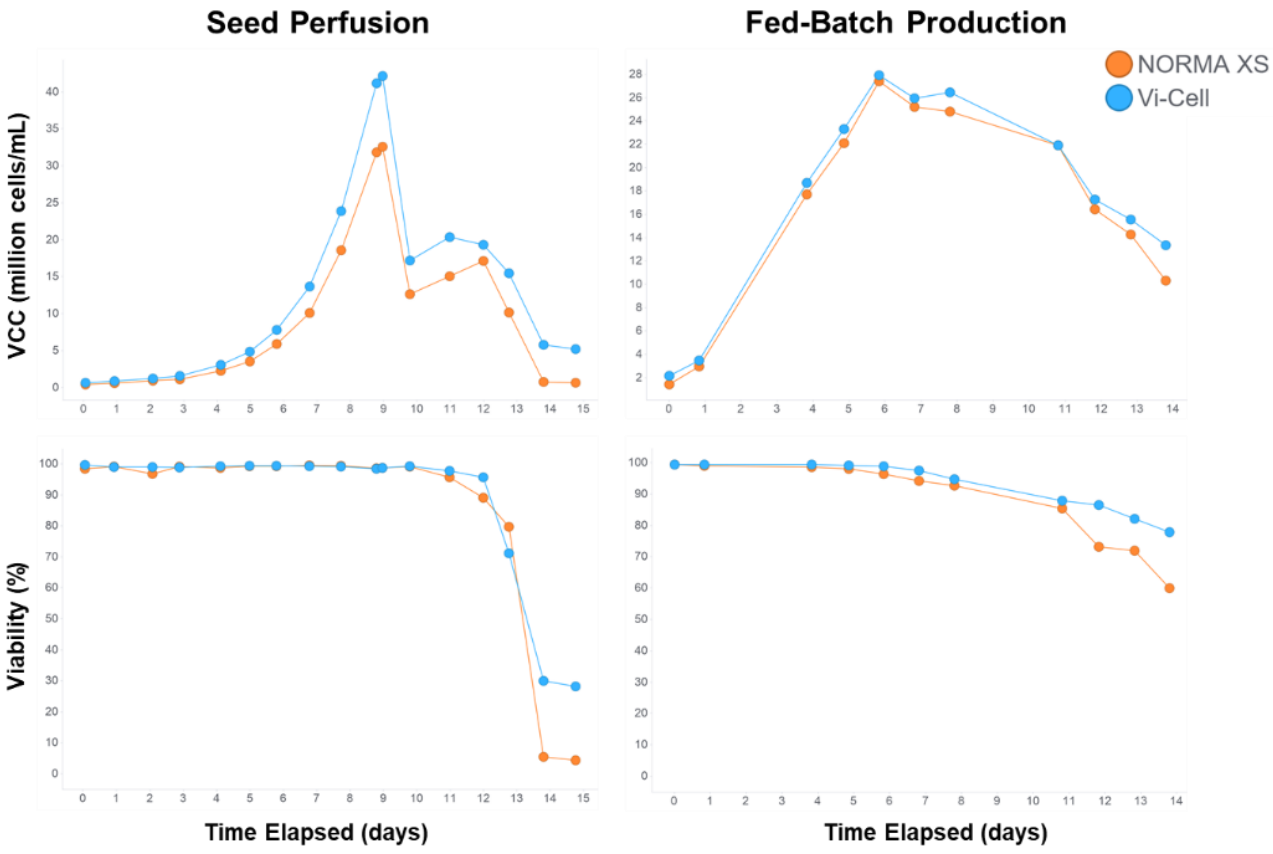
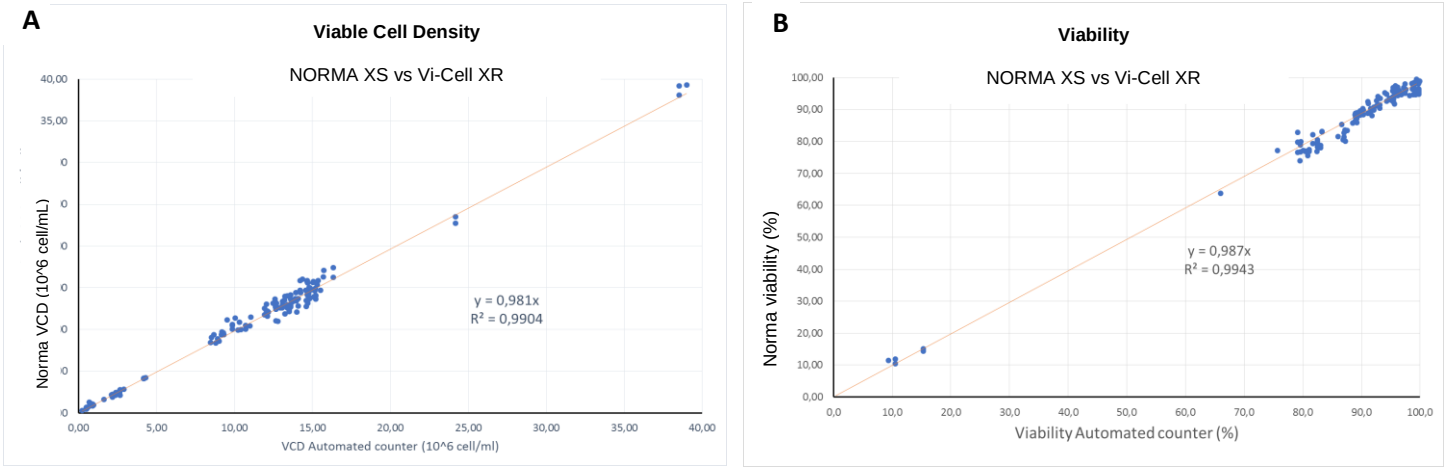


Figure 2: *the **NORMA** XS versus the Vi-Cell XR evaluation on perfusion and fed-batch processes (data courtesy of GSK)*

2. The Viable Cell Density and cell viability correlation between the Nova BioProfile FLEX, Beckman Coulter Vi-Cell XR and NORMA XS on 12 parallel fed-batch bioreactors

A second comparative survey between the Nova BioProfile FLEX, Beckman Coulter Vi-Cell XR and the **NORMA** XS was performed using 12 parallel fed-batch bioreactors up to a concentration of 15 million CHO cells. This concentration range didn't allow the **NORMA** XS performance to be pushed to its maximum, so we concentrate samples by 2 and by 4 to reach around 30 and 40x10^6 cells/mL (**Figures 3A and 4A**).

In the case of Vi-Cell XR versus **NORMA** XS test, with a concentration range up to 40x10^6 cells/mL and viability range at 75-100%, we obtained a correlation factor of 0.98 between the two compared methods. The large field of view allows the analysis of several thousand cells within a single image, keeping the statistical variability of the measure as low as 3% (**Figure 3**).



VCD Automated counter (10^6 cell/mL)

Viability Automated counter (%)

*Figure 3: Viable cell density (A) and cell viability (B) correlation between the lensless imaging technology **NORMA** XS and the trypan blue reference instrument Beckman Coulter Vi-Cell, for N=84*

In the case of BioProfile FLEX versus **NORMA** XS test, we found a ratio of approximately 1.043 between the two measurements and a coefficient of determination R^2 of approximately

0.95 with a cell density ranging from 0.2x10^6 to 30x10^6 cells/ml (**Figure 4A**). Moreover, with a cell viability ranging from 0 to 100%, we found a ratio of approximately 1.01 between the two measurements and a coefficient of determination R^2 of approximately 0.99 (**Figure 4B**).

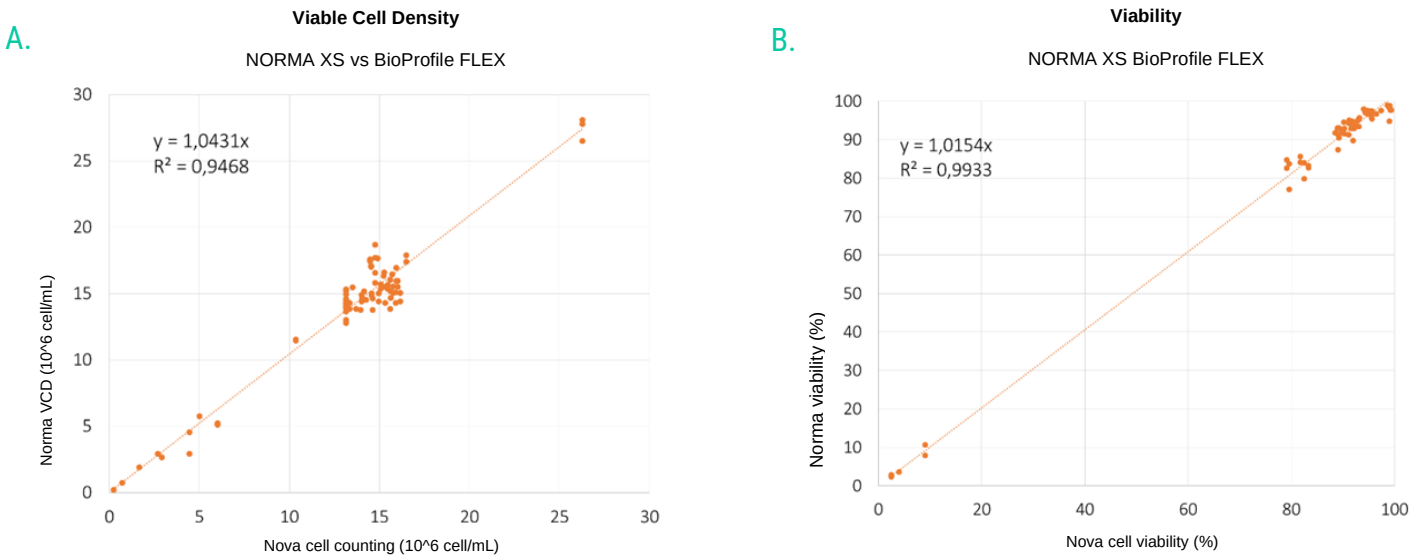


*Figure 4: Viable cell density (A) and cell viability (B) correlation between the lensless imaging technology **NORMA** XS and the trypan blue reference instrument Nova BioProfile FLEX, for N=84*

So, these two comparative studies show that the **NORMA** XS measurements agree very well with the reference techniques Vi-Cell XR and BioProfile FLEX.

3. High reproducibility of the NORMA XS

Moreover, we assessed the repeatability of our method with two tests. First, we measured the same sample 12 times with the same **NORMA** XS; and then, we measured one sample on several **NORMA** XS. These tests can therefore affirm the very good repeatability of our devices.

REPEATABILITY	CV (%)
Single sample injected in 12 different chambers and measured with 1 NORMA XS	3,23
Single sample injected in 1 Chamber and measurement with 5 separate NORMA XS	3,54

Conclusion

The **NORMA** technology is capable of accurately monitoring VCD and viability with a combination of significant advantages like low sample volume, label free detection, quick measure, simple device which let us think that it is a good candidate for very small-scale bioreactor and high-throughput measures. Its high repeatability is also a key parameter in the effort to narrow batch to batch deviations. Moreover, the **NORMA** XS measurements for cell concentrations up to about 40x10^6 cells/mL and cell viability percentage ranging from 0 to 100%, agree very well with the reference measurements Nova BioProfile FLEX and Beckman Coulter Vi-Cell XR. The coefficient of determination R^2 = 0.99 demonstrate that the lens-free microscopy **NORMA** XS is an efficient optical technique to perform cell counting and cell viability assay at large cell concentration. We envision **NORMA** to become the future method of choice for on-line monitoring of parallel suspension cell culture. It will be a perfect tool for process control in fed-batch or perfusion mode in single-use bioreactors or traditional steam sterilized vessels. It can certainly become the first VCD measurement technique from cell line engineering to process development, to pilot scale and to manufacturing scale.

Acknowledgements

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