A NEW VIABLE CELL COUNT AND VIABLITY LABEL-FREE METHOD FOR HIGH-THROUGHPUT CELL CULTURE MONITORING

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INTRODUCTION

Monitoring cell density and viability in mammalian bioreactor cultures is a critical step with a number of remaining challenges, accentuated by the evolution of high throughput cell culture technologies. Traditional measure for cell count and cell viability still rely on sampling and staining protocols, such as the Trypan Blue exclusion method, that are performed at least once a day. While automated cell counters have reduced the original manual method's statistical error, sampling of small scale bioreactors is nevertheless problematic due to significant sample volume limitations. High throughput cultivation systems add additional challenges with the need for rapid measurement. **Lensless Imaging Technology** is a new breakthrough method for accurately and precisely determining cell concentration and viability without staining.

At first we demonstrate, how this measurement technology correlates with a reference counter from different bioreactor samples. We assess the **very high reproducibility** of the technique with **a low sample volume of 3µl**. Finally, we demonstrate how this technology meets a major need today through integration with **high-throughput** microbioreactors allowing for a **full process automation**.

SUSPENSION CELL COUNT VERSUS REFERENCE

We perform the comparison of cell counts and viability determination between the Lensless Imaging Technology and the Trypan Blue reference method. Cell count is performed on CHO cells from two different processes: perfusion and fed-batch mode. The measurements were performed every day on the two instruments, except week-ends for the fed-batch run. The results showed that the NORMA XS (Lensless Imaging Technology) trended very close to the Vi-Cell XR Trypan Blue reference method for VCC (Viable Cell Count) up to $40x10^6$ cells/mL and viability from 0 to 100% for both perfusion and fed-batch processes. The measurements with the NORMA XS were performed without dilution and using just 10 µL of cell suspension. We demonstrate very good correlation between the two cell counting methods.

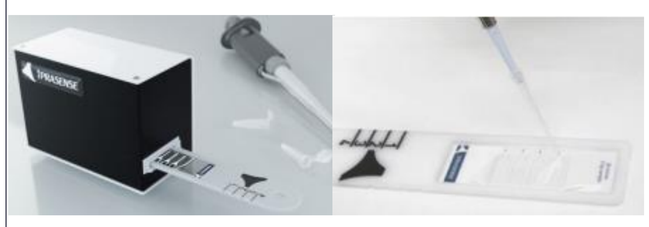


Figure 1 : loading samples on the NORMA XS

3µl sample injection in defined thickness chamber slide + NORMA slide insertion

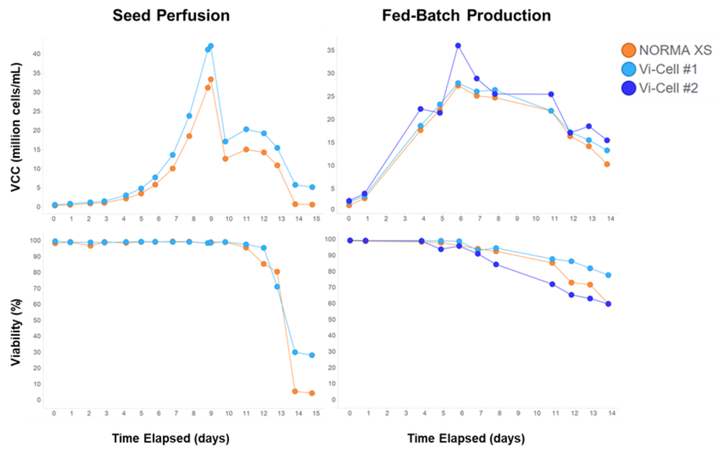


Figure 2 : NORMA XS versus Vi-Cell XR evaluation in CHO cell lines. VCC and % Viability records during the cell culture processes

HIGH THROUGHPUT CELL CULTURE MONITORING

A comparative study between the NORMA 4S and the Vi-Cell XR was performed on Ambr® 15 48-way. Six different media conditions were tested in duplicate on one CHO cell line. A sample was taken every day for each condition, except on day 1, 4 and 7. For 48 bioreactors, NORMA 4S automatically measures all bioreactors in less than 1 hour while Vi-Cell XR requires 6 hours. The cell viability and the viable cell count were measured in parallel on both instruments. Results showed a very good correlation between these two cells counters for the cell viability even when the cell viability decreases at the end of the run.

We demonstrate that the NORMA 4S was successfully integrated with the Ambr® 15 microbioreactor system and provided much needed benefits of rapid measurement and low sample size at 3 µL. Robustness of the NORMA technology was established and yielded low instrument-to-instrument variability.

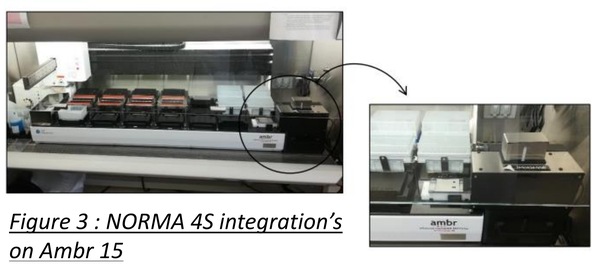


Figure 3 : NORMA 4S integration's on Ambr 15

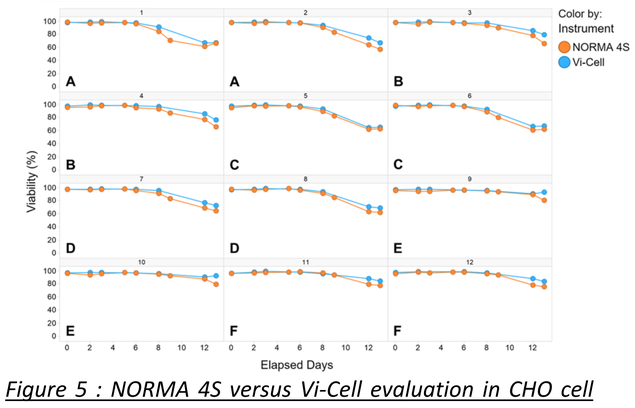


Figure 5 : NORMA 4S versus Vi-Cell evaluation in CHO cell lines. Viability (%) records during the cell culture processes

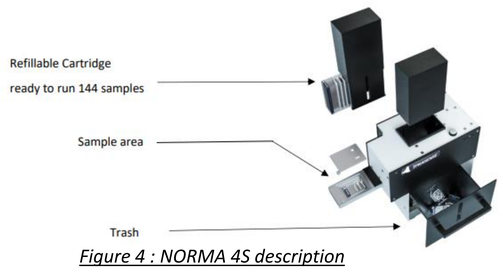


Figure 4 : NORMA 4S description

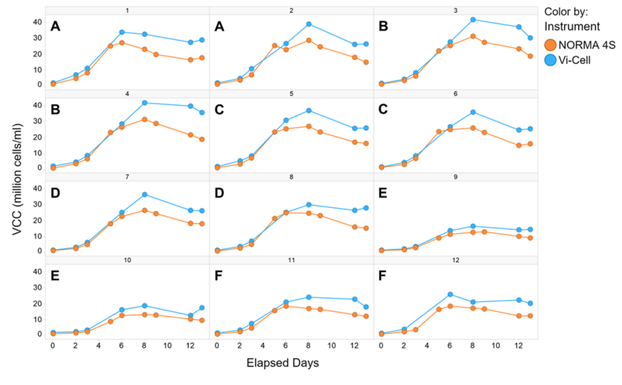


Figure 6 : NORMA 4S versus Vi-Cell evaluation in CHO cell lines. VCC (million cells/mL) records during the cell culture processes

LENSLESS IMAGING TECHNOLOGY

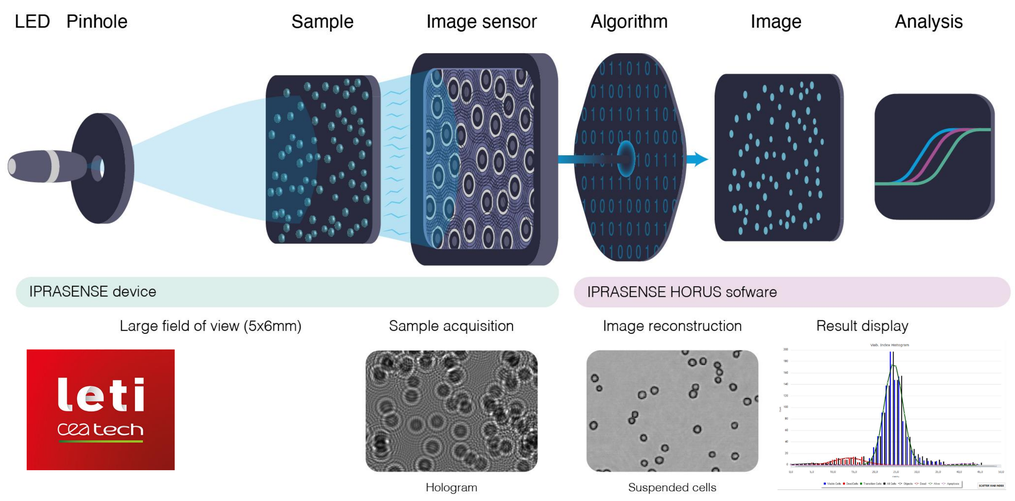
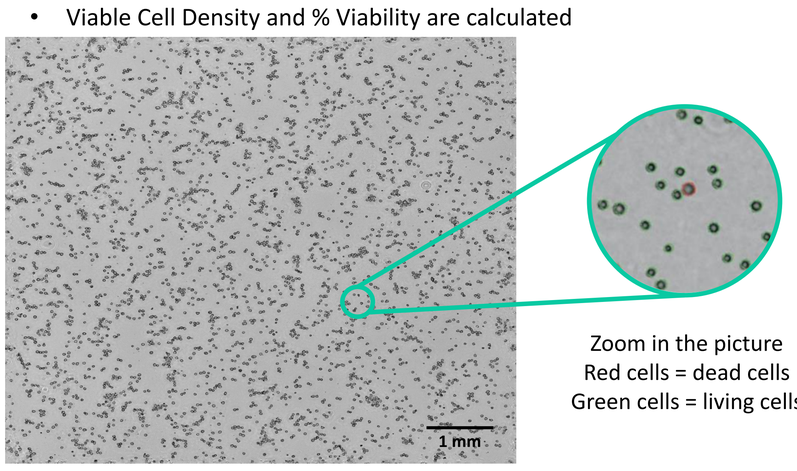


Figure 7 : Results sequence : Cells diffract light from a LED source, Holograms are created and recorded by a CMOS Sensor, Holographic algorithms reconstruct the microscopy image

CELL COUNT & VIABILITY ANALYSIS

- Cell concentration is determined from the image cell count
- Viable and dead cells have different light diffraction properties
- The viable cell holograms present a specific and significantly different pattern that can be recognized among dead cells
- Each individual cell signature is tested to assess its viability
- Viable Cell Density and % Viability are calculated



Full image field of view
displays 8656 cells (~14 million cells/ml)

Zoom in the picture
Red cells = dead cells
Green cells = living cells

KEY POINTS

- **Label Free Viability : no trypan blue**
- **Very low sample volume : 3µL**
- **High Reproducibility**
- **Extra large Field of view : 30mm²**
- **20,000 cells can be counted in a single image**

Ready for :

- **High throughput** integration to meet **future bioprocessing small volume cell culture needs**

CONCLUSION

Lensless Imaging Technology is capable of accurately and precisely monitoring **Viable Cell Density and Viability** with a combination of significant advantages including **low sample volume** use, **label free detection**, quick measure, simple device, and high number of cell analyzed which makes it good candidate **for very small-scale bioreactor** and **high-throughput** measures such as multiwell plate cell culture. We envision Lensless Imaging Technology to become the future method of choice for monitoring of suspension cells. It can certainly become the first VCD measurement technique to work from cell line engineering, to process development, pilot scale, and up to manufacturing scale.

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