Automated, closed-loop, in-line monitoring of suspension cells in bioreactor

Jérémie Barbau - OVIZIO Imaging Systems SA/NV, Brussels, Belgium

Abstract

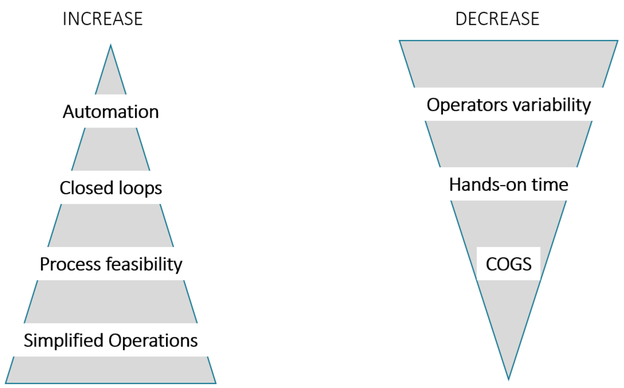
Cell-based technology is a fundamental pillar of modern biotechnology. Cell counting is one of the most fundamental metrics of it. With the development of Cell Therapy Products (CTPs), there is an increased need for robust and validated measurements for cell characterization to enable manufacturing control and a safe/high-quality product released to the patients . Our company has developed an in-line, automated microscope to monitor in real-time the suspension culture in a bioreactor. Its versatility makes it compatible with off-the-shelf stirred tanks, wave bags and others.

The cell characterization and quantification are based on OVIZIO's patented technology: Double Differential Digital Holographic Microscopy (D3HM). The microscope generates a holographic fingerprint based on 70 parameters for every cell that is imaged and feeds to a machine learning platform. Fast and accurate, the algorithms automatically discriminate living from dead cells, count and give access to in-depth quality attributes and dynamic properties of your samples, and may also provide additional information on a single cell level.

In this study we show that our in-line microscope (i) delivers a continuous monitoring of T cells culture in wave bags, (ii) counts and discriminate the viability of the T cells, (iii) gives strongly comparable Total Cell Density (TCD) and Viable Cell Density (VCD) with an off-line reference counting method (0,93> R^2 >0.99) and, (iv) tracks small phenotype changes allowing for a T lymphocytes classification (subsets, differentiation states (*still under evaluation*)).

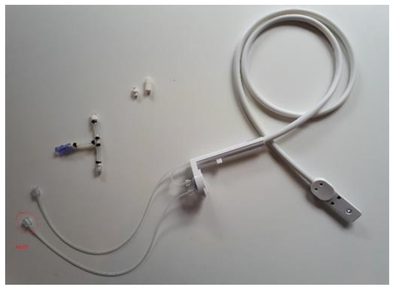
This study illustrates the robustness and reliability of OVIZIO's label-free approach for T-cell based expansion in process development or manufacturing environment. We have addressed the need to understand the biological basis for cell counting, especially when a subpopulation of cells is hypothesized to correlate with a clinical outcome.

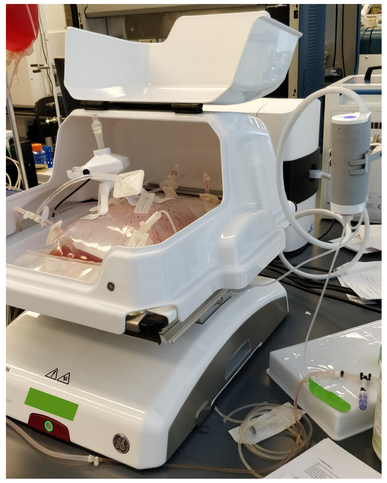
Key drivers for automation in CAR-T manufacturing



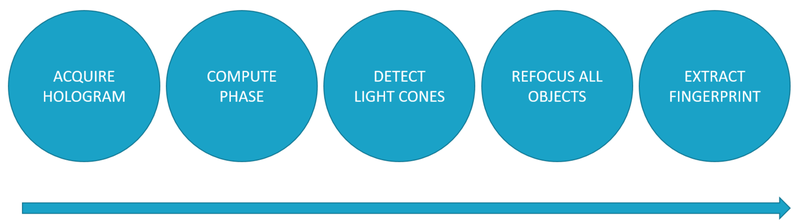
Setup

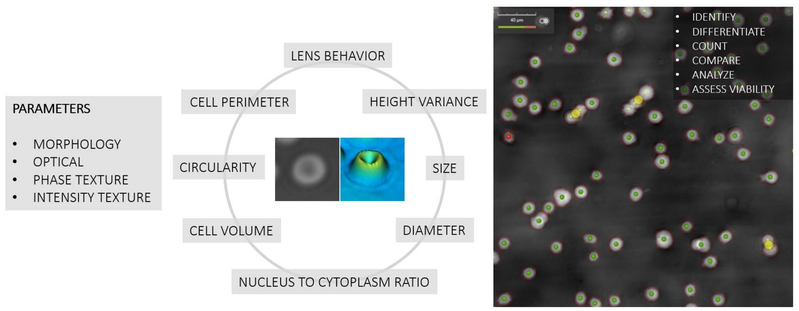
- Xuri Cells Expansion System W25 (GE)
- 2L Expansion bag, 1L working volume, pH and DO control
- BioConnect adapted for Wave-type bag connection
- CAR-T cells were inoculated at around 1x10^6 viable cells per mL
- Continuous monitoring with iLine F microscope controlled by OsOne software
- Daily sampling for off-line measurement on an automated Trypan blue cell counting device (Beckman Coulter):
  - Viability
  - Total Cell Density (TCD)
  - Viable cell Density (VCD)
  - Diameter
- Cells were grown for 3 days



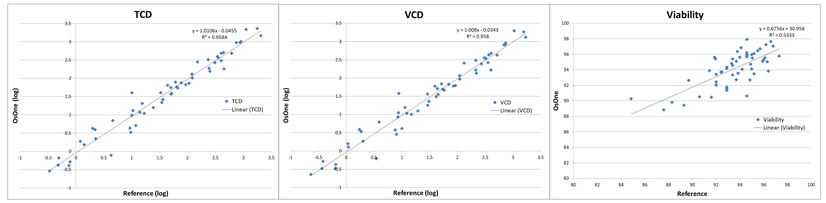


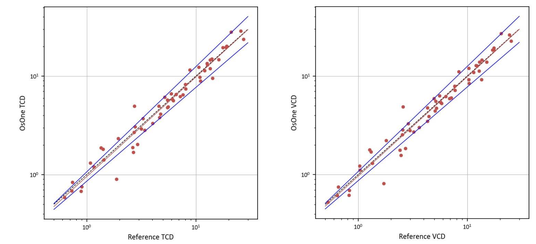
Experimental approach





Results

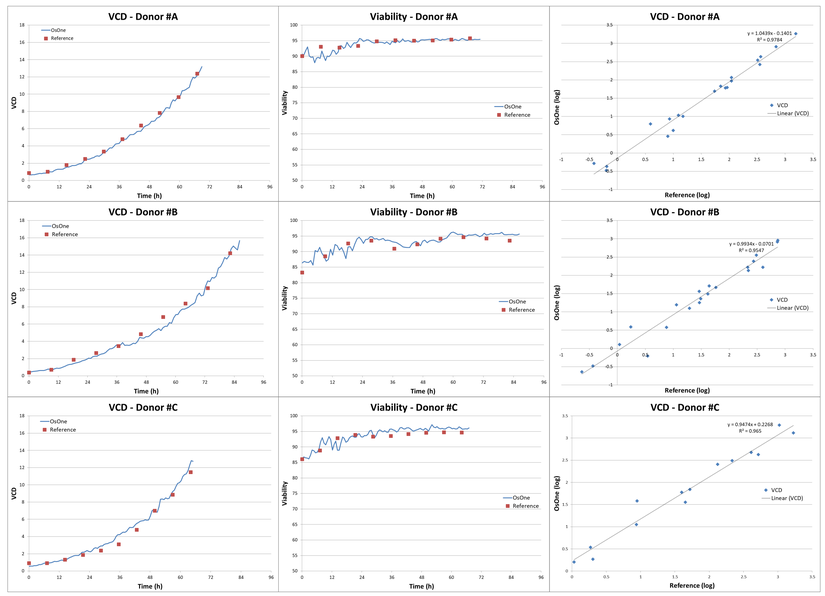




Blue lines = 95% Confidence Interval

Reference method: automated Trypan blue exclusion counting

VCD, TCD and Viability show a strong correlation with reference method.



Donor-related variation: No donor influence on the quality of the measurement.

Conclusions – Future developments

- iLine F microscope can provide **continuous monitoring** of T cells
- Cell count and viability are **strongly correlated to an off-line reference**
- There is **no influence of the donor** on the quality of the results
- OVIZIO has evidences that tracking small phenotype changes is possible, thus opening the door for **T cells subsets classification**.

Contact: Jeremie.Barbau@ovizio.com, www.ovizio.com