Automated Monitoring of CAR-T Cells in a Rocking Motion Bioreactor

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Introduction

Chimeric Antigen Receptor (CAR) T-cell therapies are showing high response rates in patients worldwide, resulting in two approved products by the US Food and Drug Administration in 2017. T-cells from a patient are removed from the blood and engineered to express the CAR to reprogram the T-cells to target patients tumoral cells. [1]

In these autologous therapies, the challenges lay in the inherent variability in starting materials and the goal of maximizing product consistency while producing a safe, pure and potent product. 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 suitable to be released to the patients. [2]

In CAR-T cell manufacturing, each handling or addition of reagents to the cell preparation generates a risk for error and for contamination that can possibly lead to the loss of a production run. A reliable solution consists of removal of open handling and implementing closed culture systems, where the cell manufacturing takes place in bags with closed tubing pathways and connections, maintaining a sterile environment.

Ovizio's patented technology, Double Differential Digital Holographic Microscopy (D3HM), is a quantitative imaging technique that allows cell monitoring in a continuous, automated and label-free set-up. No need for sampling (eliminating the risk of contamination), staining, and waiting for results generated by an off-line counter or analyzer; therefore, results are available in nearly real-time and continuous spanning the length of the culture. The platform generates a holographic fingerprint based on 70 parameters for every cell that is imaged, and feeds this data to a machine learning platform that can be trained to identify cells and discriminate between cell types. 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 application note, the use of the iLine F, manufactured by Ovizio Imaging Systems, for the monitoring of T-cells as they grow in a rocking motion bioreactor is described. The iLine F delivers reliable measurements of viability, cell density, diameter evolution and specific cellular critical quality attributes, allowing for real time in-process controls. Typically, the iLine F (i) provides a continuous monitoring of T cells culture in wave bags, (ii) counts and discriminates 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 (R^2 <0.95) and, (iv) tracks small phenotype changes allowing for a T lymphocytes classification (subsets, differentiation states (*still under evaluation*)).
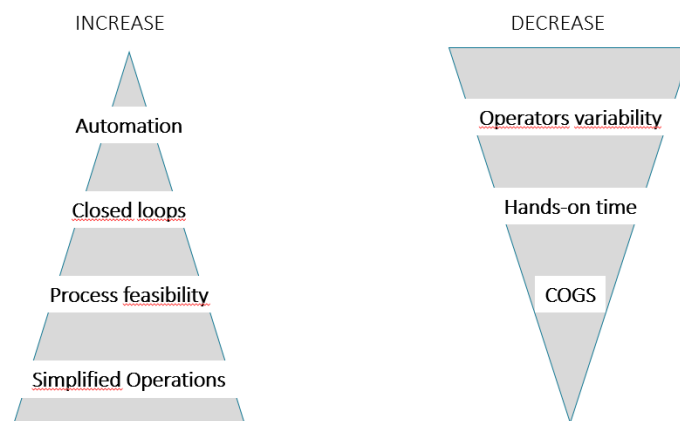

Figure 1. The needs and expectations associated with manufacturing of Cell Therapy Products.

![OVIZIO IMAGING SYSTEMS logo]

Ovizio's technology

Digital Holographic Microscopy is the scattered light beam from an illuminated object that interferes with a reference beam on a CCD camera allowing for a 3D numerical reconstruction of that object. Double Differential Digital Holography (D3HM) (Figure 2) is an evolution of this base technology that enables the use of a partial coherent light source (low power, non-invasive LED light, e.g.) resulting in improved image quality as well as an important size reduction of the instruments. A patented method is used to discriminate live from dead cells: a living cell containing cytoplasm is spherical and will create an out of focus light cone, whereas a dead cell losing membrane integrity and cytoplasm will diffuse light (Figure 2). One of the most important features of holographic microscopy is the capability of refocusing out of focus objects post acquisition. This makes the platform extremely suited of tracking objects in suspension.

The iLine F microscope acquires both intensity and quantitative phase contrast information of a microscopic sample allowing the automated extraction of 70 parameters for each object captured- the object being a cell (dead or alive).
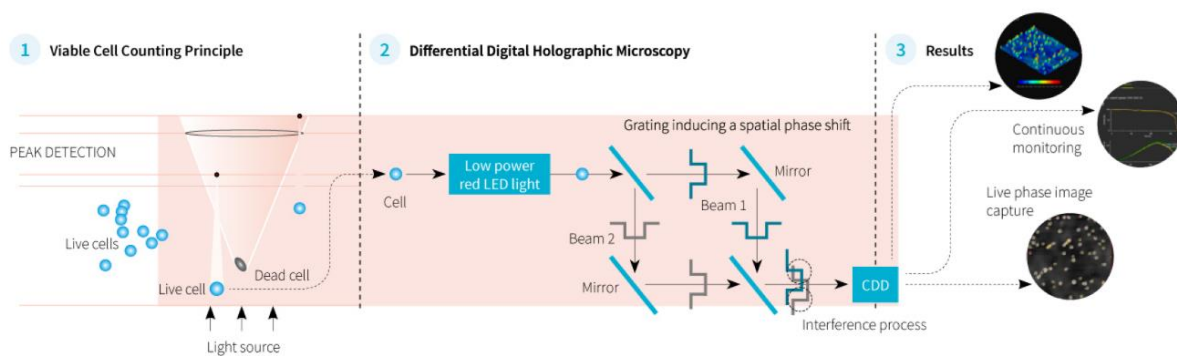

Figure 2. Ovizio's Double Differential Holographic Imaging Technology. *Live or dead cells are the objects (1) that pass through the light scattering and interference process (2) resulting in the 3D reconstruction and quantitative phase data (3).*

The iLine F can perform continuous suspension cell monitoring in rocking motion (wave) type bioreactors via an adapted setup (Figure 5). The disposable BioConnect sampling probe in combination with a reusable pump is a closed loop that pumps cells out of the wave bag through a flow cell that inserts into the iLine F microscope, holographic data is then acquired, and cells flow back into the wave bag.

The holograms are analyzed by OsOne, the monitoring software part of the iLine F microscope and a holographic fingerprint is generated for every cell found within the hologram. The fingerprint is then used by a machine learning platform trained to identify, count and analyze the sample. Viability, transfection kinetics and other cellular parameters can be detected by the platform.
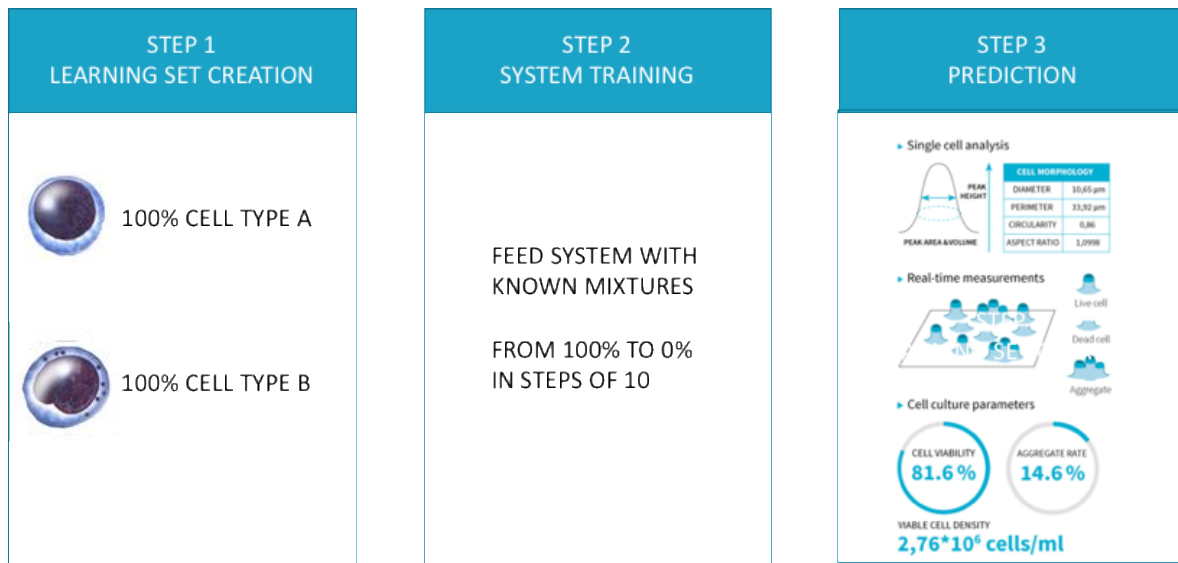
| STEP 1 LEARNING SET CREATION | STEP 2 SYSTEM TRAINING | STEP 3 PREDICTION |

Figure 3. *Machine learning principle.*

The system can be trained to discriminate and count different subpopulations by observing pure populations (100% population "A") and then observing known mixtures of that same subpopulation in incremental steps (90%, 80%, 70%, ... 10%, e.g.). The system can either automatically, or assisted by supervised learning methods, define which parameters are best to discriminate the sub populations.

Materials

- iLine F D3HM device
- Single use BioConnect monitoring probe
- Single use adapter for disposable bags
- Reusable pump engine
- OsOne Software version 5.12
- GE Healthcare Xuri Cell Expansion System W25
- GE Healthcare Cellbag 2L DOOPT II, pHOPT and Perfusion (reference 29-1054-98)
- An offline reference counting method making use of fluorescent dyes

Methods

The BioConnect, Ovizio's disposable sampling probe for bioreactor monitoring is directly connected to a disposable cell culture bag. Two silicone tubes that terminate with C-flex and male Luer locks are used to connect to the wave bag, either by welding or under sterile conditions (within a LAF hood).

The BioConnect was sterilized in an autoclave for 20 minutes at 120°C and connected aseptically to the cell culture bag using welding.

The inlet tubing of the BioConnect is connected to the harvest line in order to take advantage of the dip tubing in the cell culture bag while the outlet can be connected to the sampling port. Sampling was still possible by adding a single use (sterilized by autoclave), T-shaped adapter between the sample port and the BioConnect.
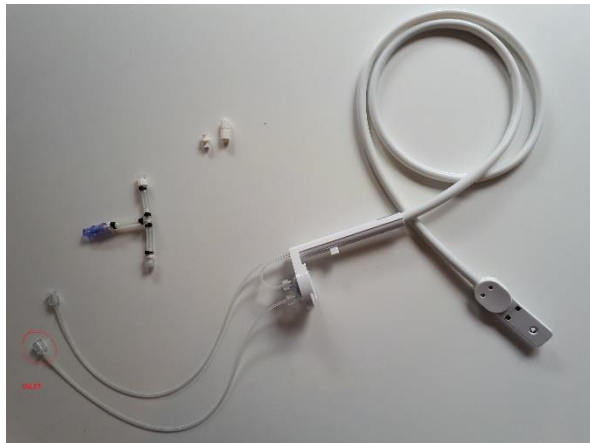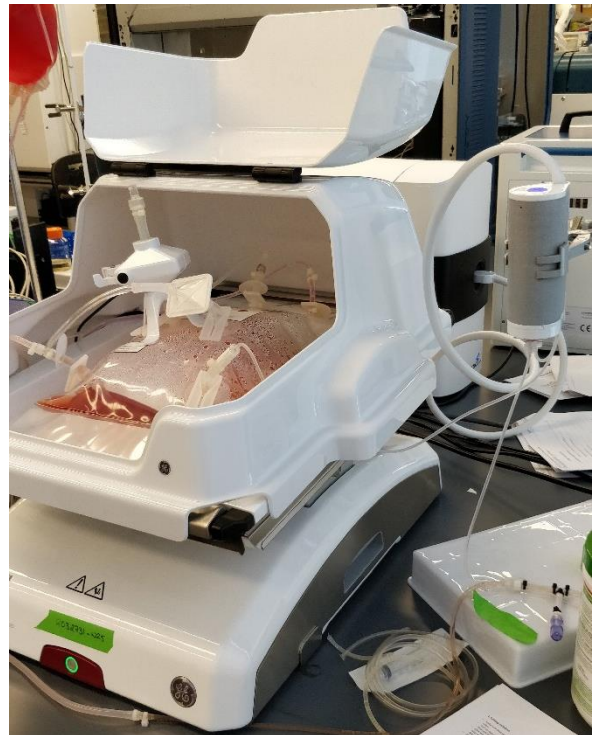
Figure 4. Modified BioConnect.

Figure 5. Complete wave bioreactor setup.

Once the assembly finalized, OsOne software was launched and a wizard designed to guide users through the selections of the proper cell type, and the number of data points to be acquired during the run, was completed.
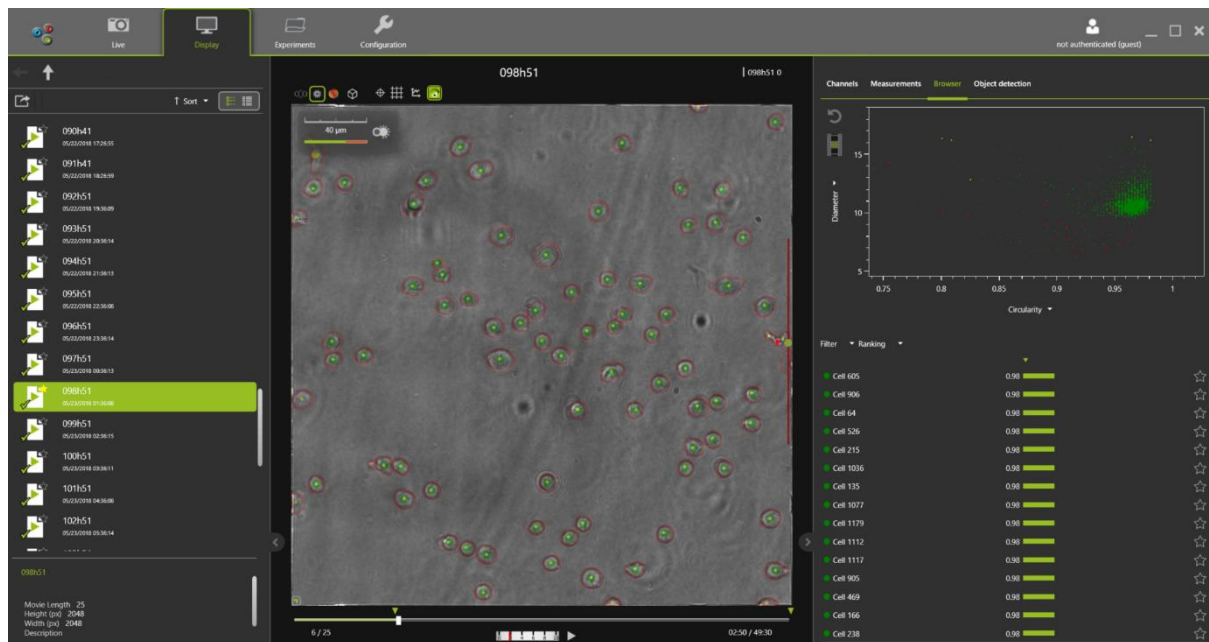

Figure 6. OsOne software.

OsOne is an all-in-one software that:

- controls the iLine F microscope
- acquires holographic fingerprints of each individual cell within the wave bag
- computes the results (data analysis)

Cell density, viability and the growth curves (viable and total cell density) are displayed on the screen for easy viewing while operating. The bioreactor was inoculated with $1x10^6$ viable T cells/ml. For this experiment, the iLine F has been set to acquire holograms every 2 minutes and computes a data point every hour. At low cell densities the system automatically doubles the number of images captured to increase statistical relevance. For every single cell imaged by the platform, 70 parameters are recorded and available for additional post processing if required.

RESULTS

OsOne plots results graphically on the screen in nearly real-time (Figure 6). During the complete run, images of the cells are acquired every two minutes. Data can be exported in multiple formats and archived for future analyses.

During the experiment data was acquired for up to 4 days continuously by the iLine F microscope and was compared to results generated by the off-line reference instrument. On average, two manual samples were acquired for off-line analyses per day.
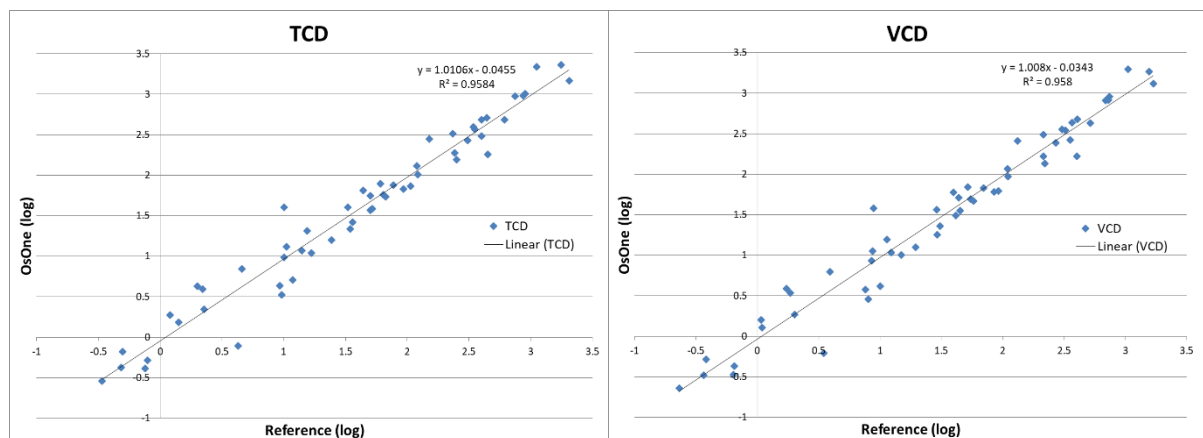

Figure 7. *Total Cell Density and Viable Cell Density compared to the off-line reference method.*

Figure 7 illustrates Total Cell Density (TCD) and the Viable Cell Density (VCD) compared to the reference method used. After plotting the data points, the correlation coefficient (R^2) was calculated and resulted in a value of 0.96. This measurement represents the actual deviation between the compared methods with respect to a linear correlation assumption, that is, if cell densities grow by a factor of two, then both methods are expected to report a factor of two. A perfect correlation results in an R^2 value of 1, though, it does not indicate to whether the absolute measurements are the same.
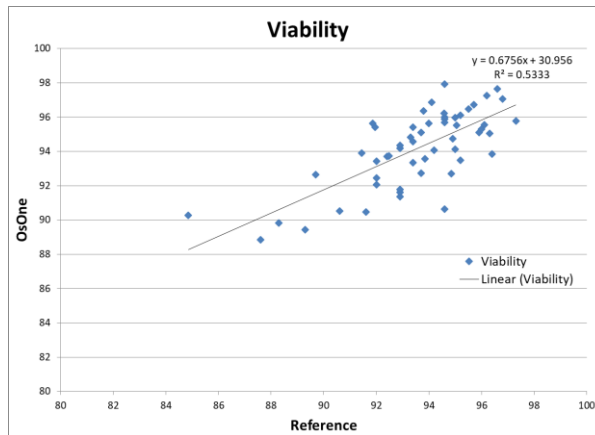
Figure 8. Viability compared to the off-line reference method.

Figure 8 illustrates Viability as provided by the iLine F compared to the reference method used. After plotting the data points, the correlation coefficient (R^2) was calculated and resulted in a value of 0.53. This value is lower compared to the value obtained when comparing TCD and VCD with the reference method. It can be explained by the fact that viability stays around 95% during the course of the culture. Indeed very few data points can be found below 90%. It results in the values being part of a cloud of points instead of being distributed along a line. As a consequence, fitting a regression line is much more complicated hence the low value for the correlation coefficient. However, the discrepancies between the values generated by the iLine F and the reference method are minor: mostly within 5% of variability.
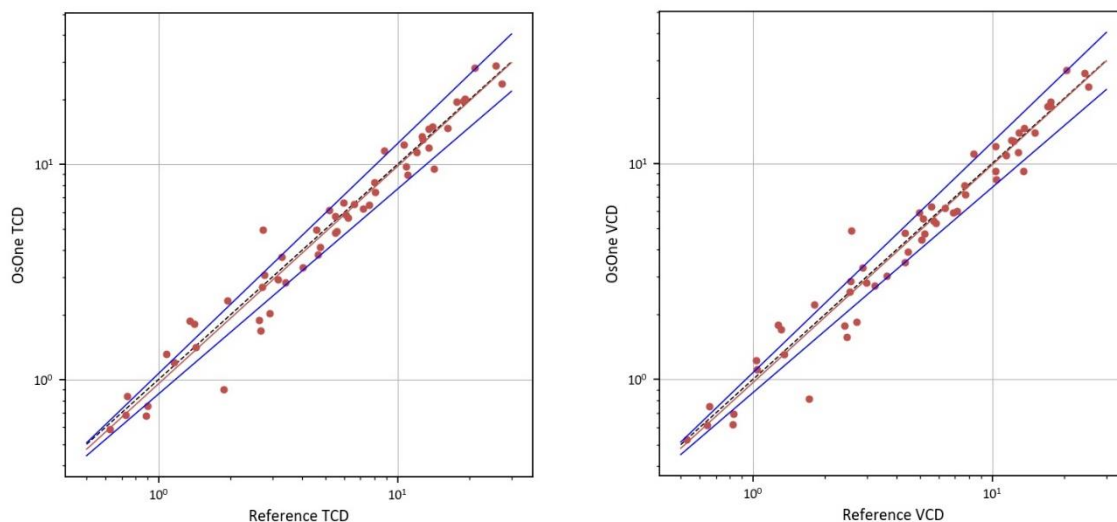

Figure 9. Total Cell Density and Viable Cell Density compared to the off-line reference method within a 95% confidence interval.

A 95% confidence interval has been fitted on the Total Cell Density and the Viable Cell Density, showing that most of the values are within this confidence interval.
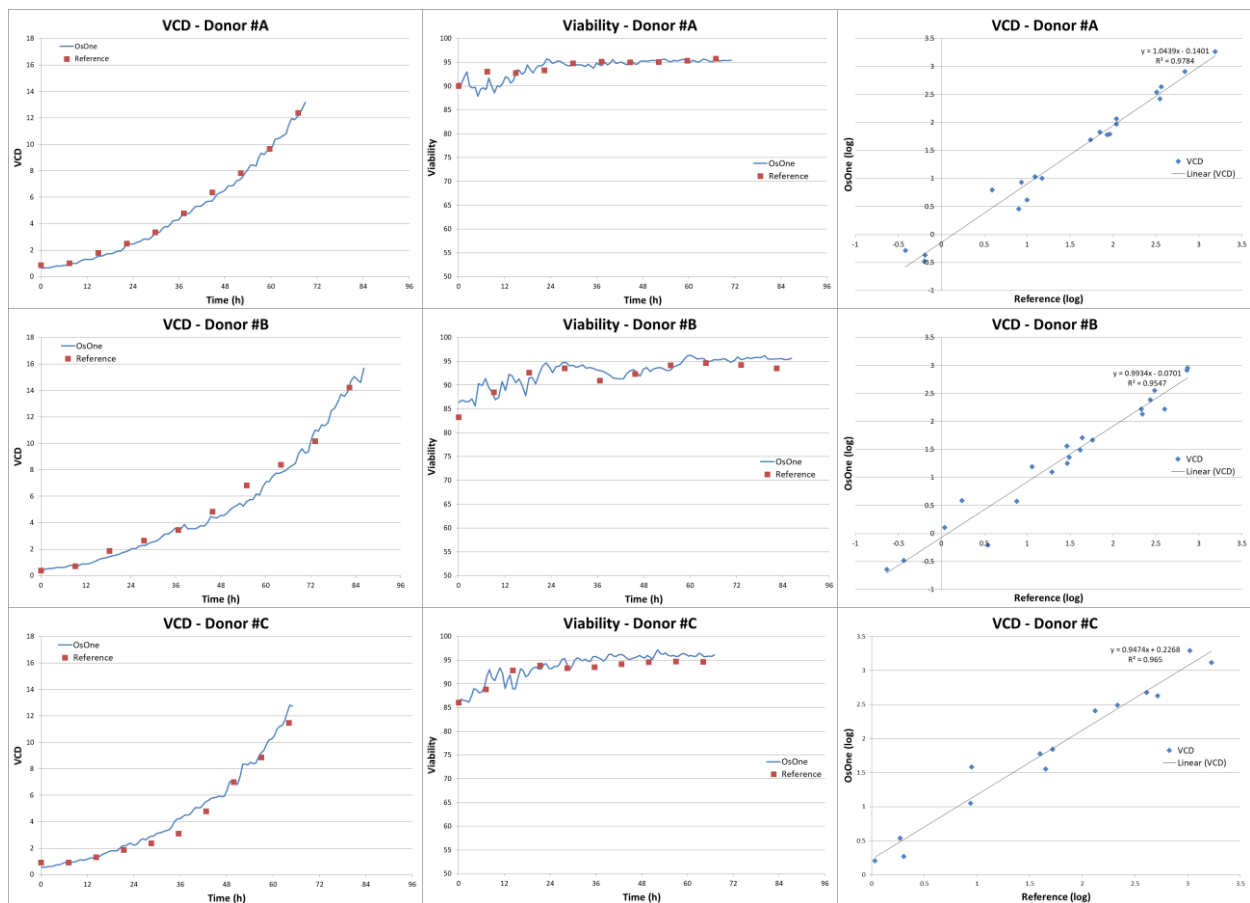
Figure 10. *Donor-related variation shows that there is no influence from the donor on the quality of the measurement.*

Donor-related variations have been investigated. Figure 10 gives an overview of 3 different donors. Differences in the cell growth pattern can be seen while the correlation between the data provided by the iLine F and the reference method remain very good for each donor. It shows that the system is able to produce reliable data, independently of the donor.

It has to be noted that at the start of the run, there are less cells and thus more empty images, therefore, we observed some variability in the results when compared to the first three off-line data points. After 24 hours the correlations improved as the density increased with culture growth. Improvements have been made in the OsOne software to acquire more images at low densities to mitigate this limitation.

CONCLUSION

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 and characterization, especially when a subpopulation of cells is hypothesized to correlate with a clinical outcome.

The iLine F, in-line technology combined with the machine learning based analysis software OsOne, delivers results that are nearly equivalent to traditional off-line methods. Moreover, it provides additional information on a single cell level for continuous monitoring of CAR-T cell cultures. As the system is automated, there is also no need for an operator to sample the cell culture bag, thus eliminating operator-related variability and reducing operational costs.

The iLine F and adapted BioConnect disposables can be used as a Process Analytical Tool in a closed manufacturing environment with improved product characterization maintaining sterility throughout the cell expansion process. The instrument can be connected to the manufacturing control environment via OPC and is CFR 21 part 11 compliant. Each run is archived, and data can be accessed for analyses or regulatory purposes.

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