iLine F

OVIZIO IMAGING SYSTEMS

Automated Suspension Cell Counting in Stirred Tank Reactors Versus Traditional Methods

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Abstract

Cell counting in large scale suspension cell cultures often relies on sampling followed by a staining procedure. An estima-tion of the cell concentration and cell viability is traditionally performed once a day using a Trypan-Blue cell exclusion method or another. These off- line techniques require manual operations, with the stained samples being destroyed at the end (creating toxic waste). It often requires weekend work too.

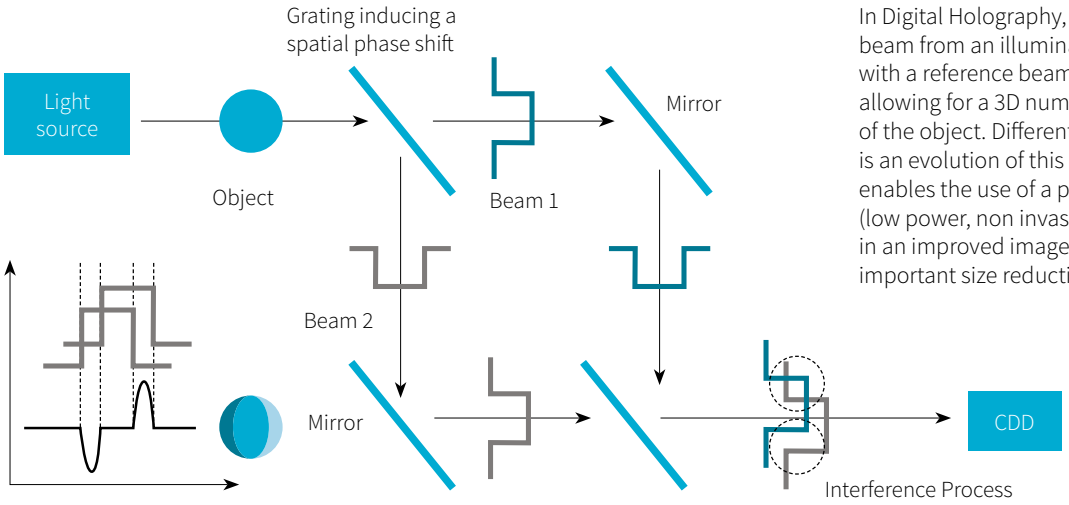
Differential Digital Holographic Microscopy (DDHM) is a new quantitative imaging technique that allows **cell counting as well as cell viability monitoring in a continuous, label-free set-up**. No need for sampling (eliminating the risk on contamination), staining and waiting for the results generated by an off- line counter: your results are available in nearly real-time and this over the whole run.

The operator can track the total cell density (TCD) plus the cell viability (VCD) at any time, while the software OsOne plots the **cell growth curve live** on the screen. Moreover, OsOne also shows real-time images of the cells, offering experienced operator a particularly convenient tool to check the condition of his cell culture: "**Seeing is believing**".

The holographic microscope used for this experiment was the OVIZIO iLine F. This device has been intensively **cross-validated versus the Vi-Cell (Beckmann Coulter) and our manual counting method**. The results are described in this application note. The iLine F has also been validated versus other cell counters like the Cedex (Roche) and the CASY® (data available soon).

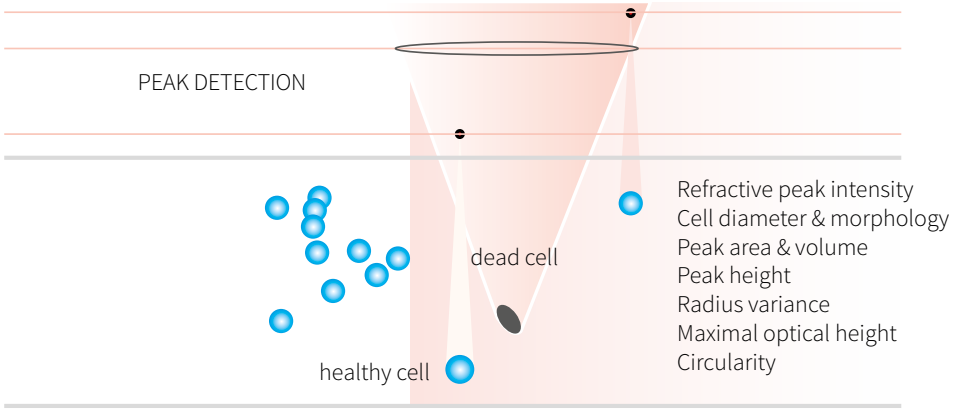
Working Principle

The technology is based on the use of a Mach-Zehnder interferometer



In Digital Holography, the scattered light beam from an illuminated object interferes with a reference beam on a CCD camera allowing for a 3D numerical reconstruction of the object. Differential Digital Holography is an evolution of this base technology that enables the use of a partial coherent light (low power, non invasive LED light) resulting in an improved image quality as well as an important size reduction of the instruments.

Figure 1 Differential Digital Holographic Microscopy Principle



Discriminating live from dead cells based on their 'holographic fingerprint': when illuminated, healthy cells are creating light cones (lens effect), while dead cells show scattered, less intense focalization peaks. Other parameters are also taken into account (eg. cell diameter).

Figure 2 Viable Cell Counting Pinciple

The iLine F & the BioConnect for In-Line, Non-Invasive, Label-free Cell Monitoring

The iLine F can perform continuous suspension cell counting in most stirred tank bioreactors.

Through applying a closed loop set-up, cells are being pumped out of the bioreactor into the BioConnect. Cells travel through a flowcell where holograms are being captured and then flow back into the reactor. The BioConnect is an innovative, auto-clavable & disposable pumping system working like your heart, not affecting the cells .

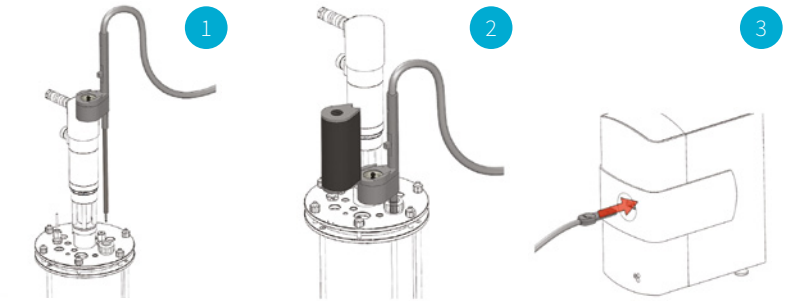
The holograms are continuously analyzed to compute cell density and cell viability in nearly real-time.

Figure 3 iLine F System

Materials & Methods

- iLine F DDHM device with single-use autoclavable BioConnect™ fluidic unit plus a reusable pump engine
- OsOne Software version 3.8 (already includes the required cell detection algorithms)
- Applikon 3 liters glass bioreactor controlled by ez-Control
- CHO cells inoculated at 0.3x10^6 viable cells/mL in CD-OptiCHO™ medium (Life Technologies)

Figure 4 Set Up & Start Up Guide



Procedure:

- Assemble bioreactor & BioConnect™ (Figure 4 - 1)
- Autoclave together
- Prepare your bioreactor (media, cells)
- Connect bioreactor and controller
- Add BioConnect™ pump engine (Figure 4 - 2)
- Activate the BioConnect™ by inserting the cartridge into the iLine F (Figure 4 - 3)
- Define or select your experiment in OsOne
- Press start.

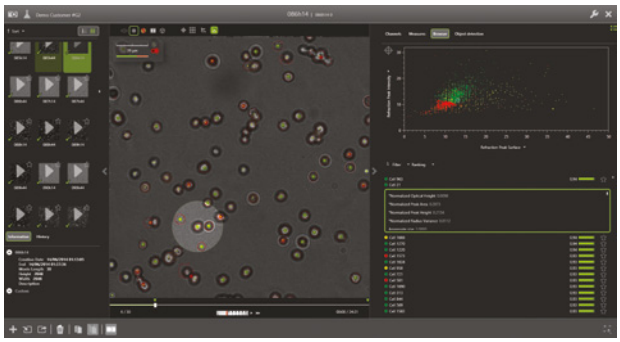


Figure 5 OsOne Software Interface

Typical results with overlay on cells: The browser shows the image acquisition of the cells and a dot plot of the cell population characteristic: green dots represent living cells, red dots are cells classified as dead and clusters are identifiable by the yellow dots.
A simple click either in the dot plot (Figure 6) or in the image (Figure 5).

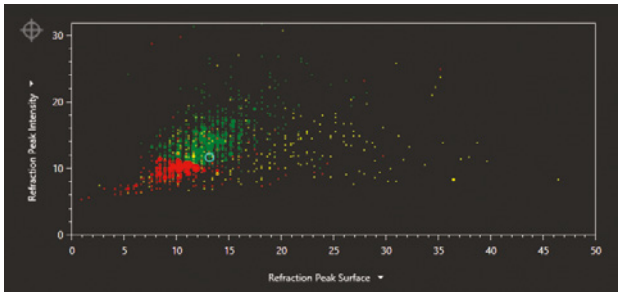


Figure 6 Cell Browser

The dot plot represents the total identified cell population, a helpful tool when studying for example shifts in population characteristics.

Image acquisition and analysis: OsOne Software

OsOne is **an all-in-one software, developed at OVIZIO**, that:

- Controls the iLine F microscope
- Acquires holographic fingerprints of each individual cell
- Computes the results (data analysis)

This advanced, yet easy to use, software captures real time 3D images with quantitative data allowing for automatic cell counting and other specific measurements. **OsOne allows the user to focus on the result of a measurement while offering all the tools required for a thorough investigation of underlying data.**

Cell density, viability and the growth curves (viable and total cell density, are displayed on the screen for easy viewing while operating.

Compared to classical light microscopy, Differential Digital Holographic Microscopy offers:

- A greater depth of focus: 100x (applying the same magnification)
- The ability to refocus images post acquisition
- The collection of quantitative phase information (optical density), covering the shape and density of an object. This quantitative phase parameter (not captured by the human eye) is of major advantage in numerous applications developed at OVIZIO.

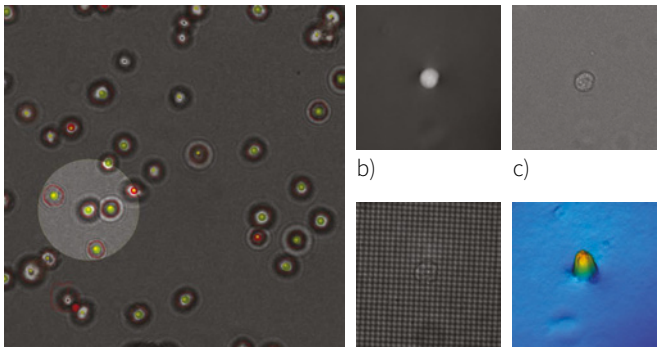
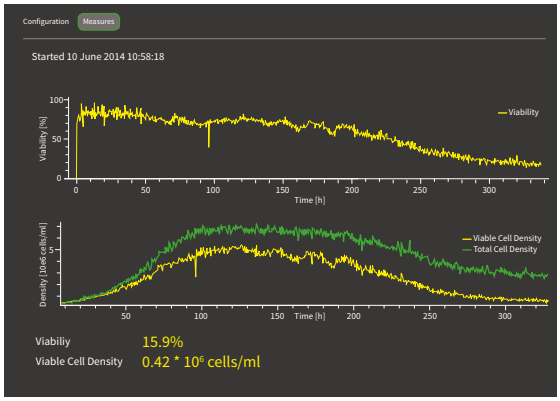


Figure 7 Multiple Holographic Views Captured by the iLine F Microscope

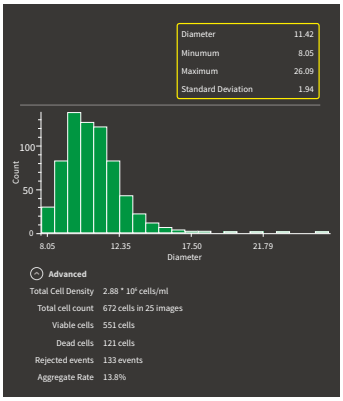
a) Cells with overlay
b) Phase image
c) Intensity image
d) Hologram
e) 3D view: the height of the peaks represent the optical height which is the optical thickness of the cells

Results

OsOne plots the results graphically on the screen, in nearly real-time and during the complete run. From the growth curves (Figure 8 a), the different phases of the culture (from the lag phase to the death phase) can be easily followed enabling for example to anticipate feeding and harvesting times. The iLine F in combination with the OsOne software is a sample and label-free method that has been found to be as reliable as the reference methods applying Trypan-Blue staining. Indeed, continuous measurements via OsOne fits well with the time-point results obtained by the Vi-Cell (an off-line counter using Trypan blue from Beckman Coulter) as well as with manual counting (Figure 9). The representation of the 'Viable Cell Density (VCD)' measured with OsOne versus the one obtained from the Vi-Cell shows a good correlation (R^2 = 0,9253). Similar results have been obtained for the 'Total Cell Density (TCD)' and the viability over time (data not shown here) and independent sources have confirmed results versus the Cedex (Roche) and the CASY (Innovatis, data from Prof. Gudermann, TU Bielefeld).



a)



b)

Figure 8 Continuous Monitoring with the iLine F ystem

a) **Over time**: Viability, Total Cell Density and Viable Cell Density are measured during the complete run.
b) **Time point**: Data can also be represented for a selected time point: here a bar chart representing the cell population repartition according to the diameter and statistic data such as average, minimum and maximum.

Figure 9 Counting Systems Comparison Over Time (OsOne, Vi-Cell & Manual Counting)

Viable cell Density was measured over time during 96 hours with different methods: the OsOne software, the automatic Trypan-Blue Vi-Cell method and the Trypan-Blue manual counting.

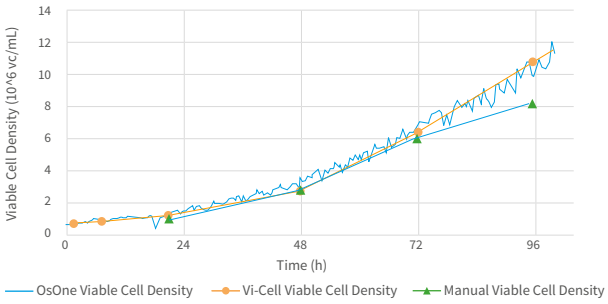
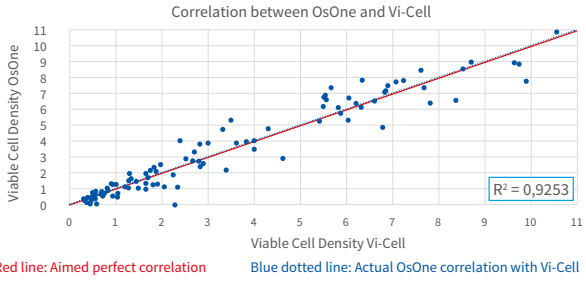


Figure 10 OsOne and Vi-Cell Equivalence

Correlation between Vi-Cell and OsOne cell count
Representation of the Viable Cell density measured with OsOne versus Vi-Cell



Conclusion

This study proves the robustness and reliability of OVIZIO's label-free approach. The iLine F in-line technology combined with the powerful analysis software OsOne delivers equivalent results compared to the reference methods. Above that it offers the advantages of a continuous, sample free method, avoiding the use of stains (often resulting in toxic waste). It also opens the door for further automation (for example through its integration in systems solutions like the BaychroMAT®, or through a link via the bioreactor controllers or even through its integration in analysis software such as Lucullus Process Information Management System).

The availability of all this data at single cell level during complete experiment, allows to envision the use of the iLine F for PAT approach. Indeed, the large amount of data produced can be used to perform various statistical analysis on the cell population, enabling to define and control critical parameters of the process to achieve validation, QC or audit for instance.

Sales and Service contacts

For more information on the product:
www.iLine-F.com

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