Continuous suspension cell culture monitoring in bioreactors using quantitative phase imaging

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

Abstract

Monitoring of suspension cell cultures often relies on sampling followed by a staining procedure. Estimations of cell count and cell viability are traditionally performed once a day using Trypan-Blue cell exclusion as a method of choice. This process involves manual operations and weekend work is regularly needed.

Quantitative phase imaging (QPI) 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 (thus eliminating the risk of contamination and the generation of toxic wastes), staining and waiting for the results generated by an off-line counter: results are available in nearly real-time over the whole run.

Compared to classical light microscopy, QPI offers:

- 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 the key advantage in numerous applications developed at OVIZIO.

QPI helps the operator to track the total cell density and the cell viability at any time, while the OsOne software plots the cell growth curve, live on the screen. Moreover, OsOne also shows real-time images of the cells, offering the experienced operator a convenient tool to look at the culture, live.

Results generated by an iLine F were compared with a reference method: the Vi-Cell XR off-line counter (Beckman Coulter), automated Trypan-Blue staining. A correlation factor R^2 of 0.982 was obtained on the Viable Cell Density demonstrating that the results obtained with QPI are in line with current reference methods.

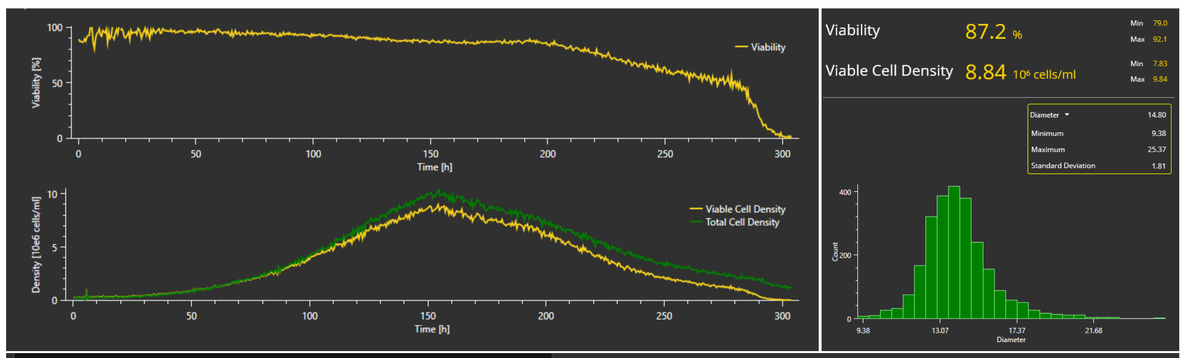
Advantages

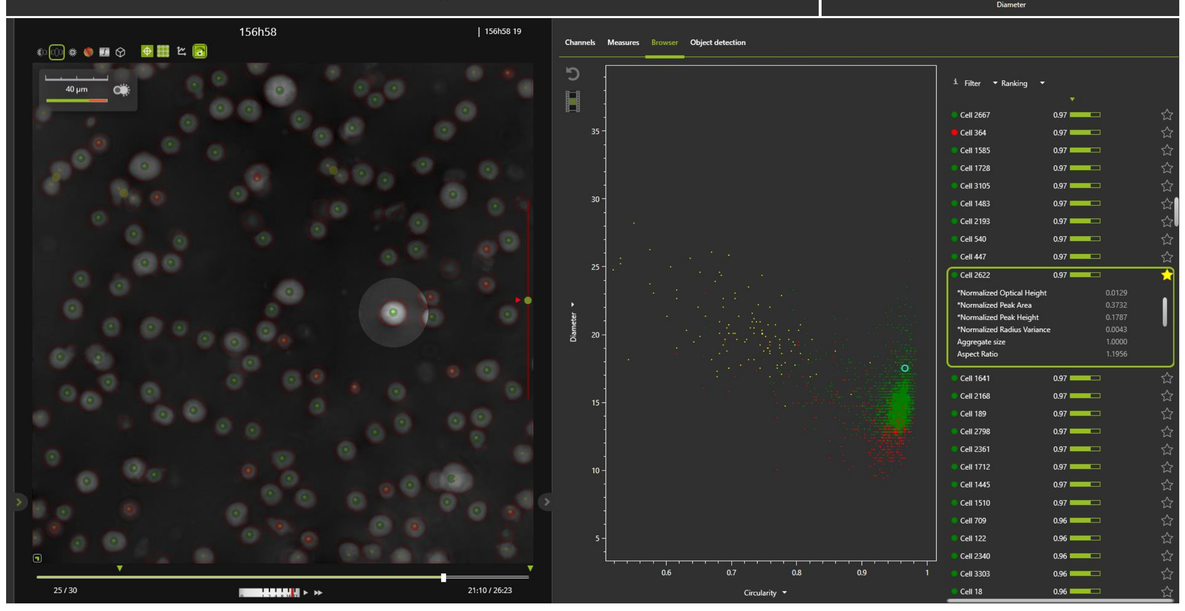
- Full traceability at single cell level
- Continuous monitoring
- User independent, improving reproducibility
- No sampling, no staining
- No toxic waste
- Ready for automation
- Increased insight in your culture process
- Adds aggregates information as aggregates are not disrupted during sample preparation
- More than just cell density and viability: up to 70 parameters are recorded

Materials & Methods

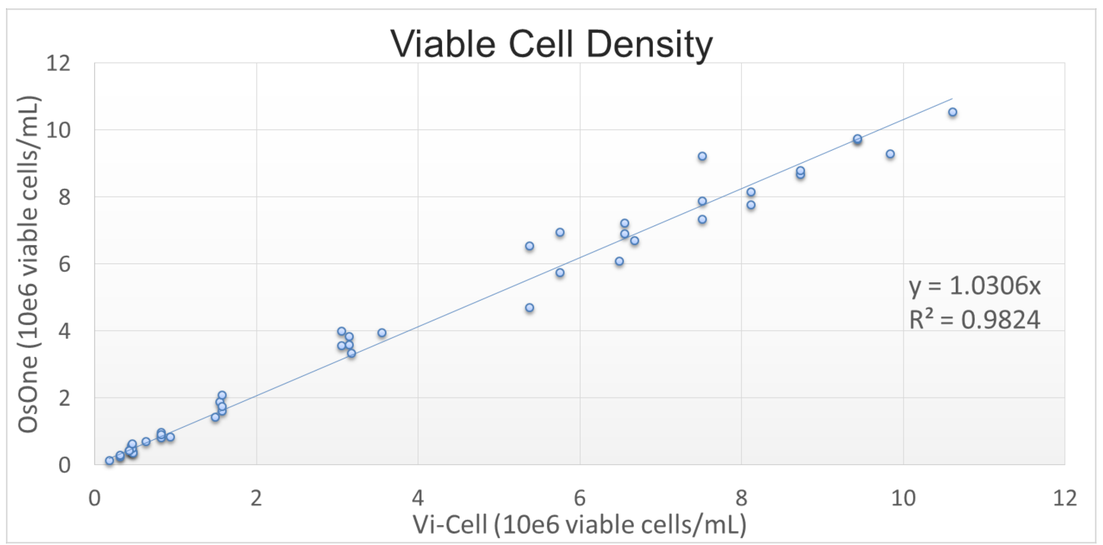
- iLine F microscope.
- **BioConnect:** autoclavable and disposable **closed-loop** interface with the bioreactor.
- Reusable pump engine with automated flushing (in case air bubbles or large aggregates are detected, patent pending).
- **OsOne Acquisition & Analysis software** (developed in-house by OVIZIO), version 5.9.
- Applikon 3L glass bioreactor, controlled by ez-Control.
- CHO cells inoculated at $0.3x10^6$ viable cells/mL in CD-OptiCHO™ medium (Life Technologies), batch culture for 12 days.

Results





Conclusions



- **Very good correlation (R^2=0.982) of the viable cell density** compared to classic staining methods applying sampling and Trypan-Blue staining. This also proves **the label-free approach is at least equivalent** to extensively validated reference methods.
- **Full data per cell is available**, for the whole experiment, for validation, QC, audit, etc.

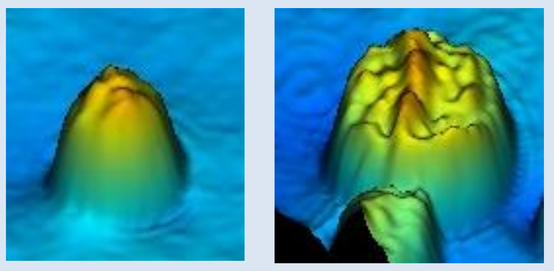
Future Developments

- The availability of full data, per cell, for the whole experiment allows **the use of the iLine F for a PAT approach**. Indeed the large amount of data produced can be used to perform various statistical analysis on the cell population, **at the cell level**, in order to define and control critical parameters of the process.
- The **iLine F can be linked with the bioreactor controller** in order to control for instance the feeding, based on the cell density, or the harvest time after a viral infection, based on the viability of the culture. The next version of the device will introduce multiplexing: OsOne will act as a **central control server** that collects data from several iLine-F simultaneously. Thus a centralized computer performs all the calculations while only the microscope occupies valuable space in the lab.
- Finally, an API (**Application Programming Interface**) is currently in development in order to integrate into other Processes Information Management Systems.

Other Applications of QPI

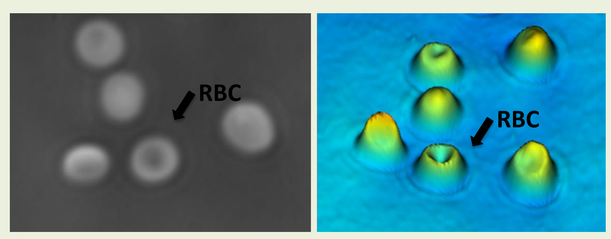
QPI has the capability to generate an optical fingerprint for any cell type. As a consequence, many other applications are possible, such as:

Counting of infected cells: when infected by a virus, cells are showing a clear optical fingerprint that can be captured by QPI. With a limited training set, a false positive rate of 8,2% and false negative rate of 7,3% were obtained.



Non-infected cell | Infected cell

Identification and count of blood cells: each blood cell type has a particular optical fingerprint linked to its shape, size and content.



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