Quantitative Phase Imaging : from cell counting to continuous monitoring of viral load in a bioreactor

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Abstract

Quantitative Phase Imaging is currently used to count and determine viability of a variety of cell types. When a cell culture process involves an infection step (e.g. Baculovirus Expression Vector System, BEVS in insect cells), it is not yet possible to continuously monitor the infection kinetics. To do so, sampling and off-line analysis are required. However, real time results are important because they can impact the way the culture is performed, for instance addition of nutrients, change of cell culture parameters, optimal harvest time, etc.

Our study shows that using a novel technology, Quantitative Phase Imaging (QPI, based on Differential Digital Holographic Microscopy), a detection system can be trained to identify infected cells, and then be used to determine the percentage of infected cells within the culture in real-time.

With these limited data sets, a false positive rate of 8.2% and a false negative rate of 7.3% were obtained. Following this, the algorithm was integrated into OsOne, a cell culture monitoring software tool, and used on different Sf9 cultures infected with the Baculovirus Expression Vector System. Results have shown that a strong correlation between offline analysis and online analysis can be achieved, confirming that the detection algorithm created is reliable and in line with standard, off-line methods.

In conclusion, the detection system was trained to extract a specific cellular fingerprint pre- and post-infection, thus allowing in-line, continuous monitoring of an infection process.

Results

Sf9 cells were inoculated in a bioreactor at a density of 0.5×10^6 viable cells, with a viability above 99%. Cells were infected with BEVS at a MOI of 0.01 after 80h of culture. Fluorescence is used as reference method for determination of infected cells density during cell culture (sampling required).

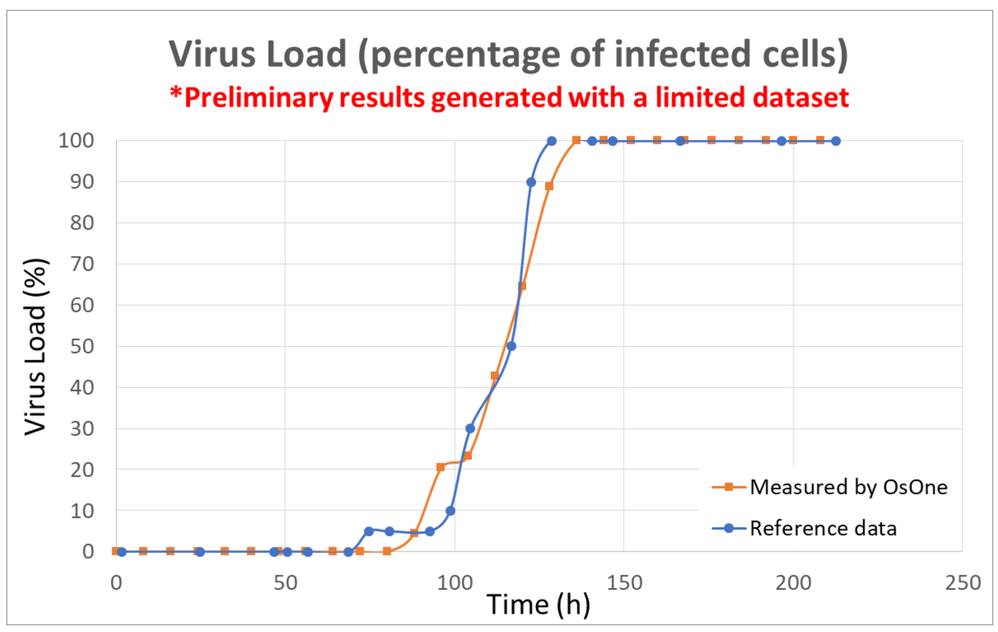
Training of the algorithm with the normal and infected cells:

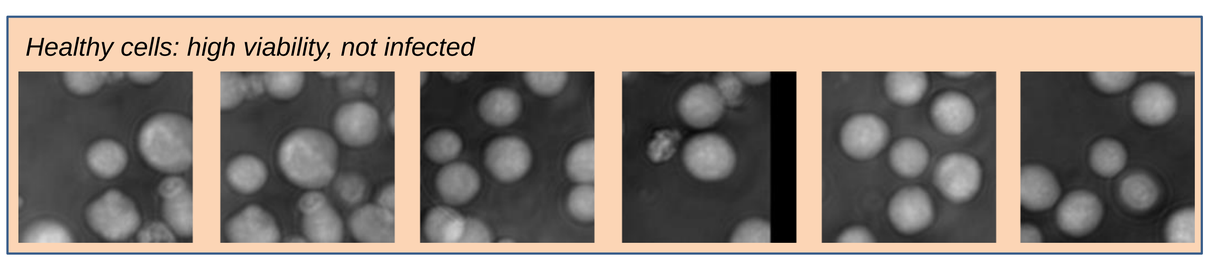
$$\begin{pmatrix} \mathbf{0.79} & \mathbf{0.21} \\ \mathbf{0.21} & \mathbf{0.79} \end{pmatrix}$$

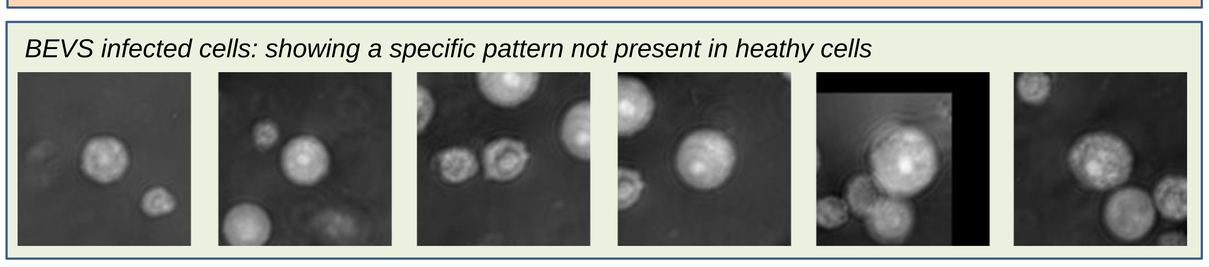
Weights of the different classes can be changed to avoid false positives:

$$\begin{pmatrix} \mathbf{0.45} & \mathbf{0.55} \\ \mathbf{0.03} & \mathbf{0.97} \end{pmatrix}$$

In that case, the algorithm has 97% chances to identify a non-infected cell correctly and a 55% chances to identify an infected cell correctly. Further "directed training" of the algorithm allowed it to reach a false positive rate of 8.2% and a false negative rate of 7.3%, with regards to infection by BEVS.



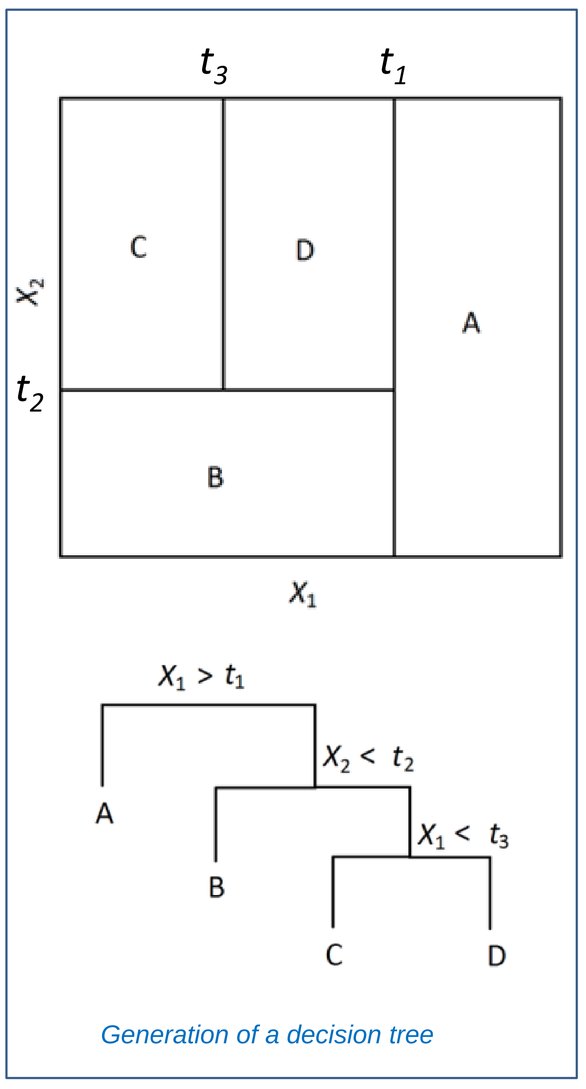




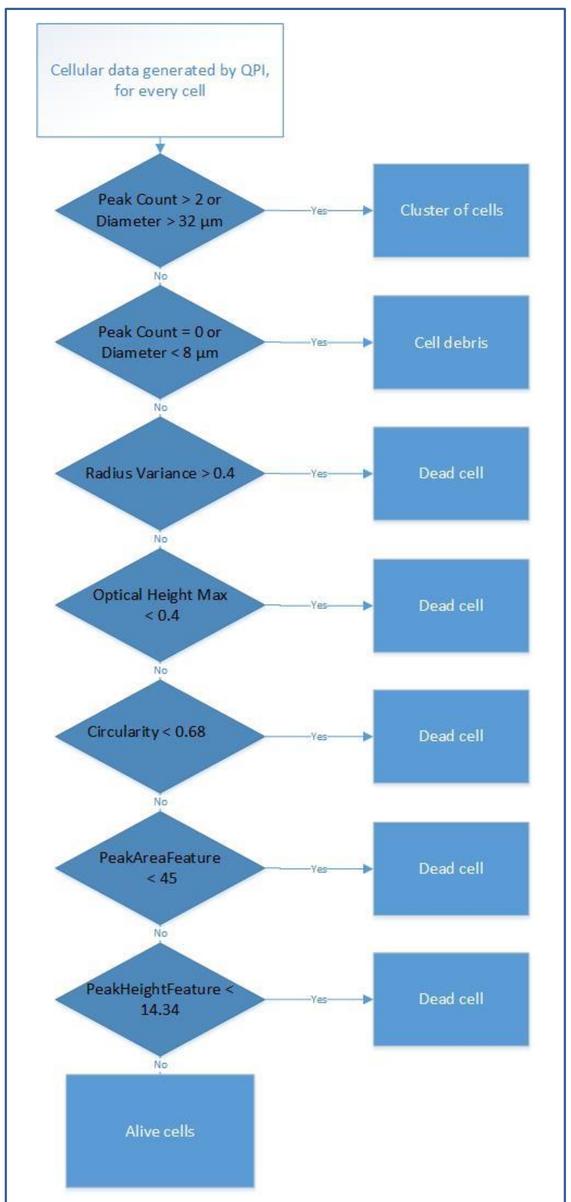
Experimental approach

For each cells, **QPI has the capability to generate 70 parameters**. These parameters are already used in OsOne (a cell culture monitoring tool designed by OVIZIO) to determine the if a cell is dead or alive and then compute the viability of the whole cell population. This specific viability decision tree has been created manually and make use only of the most basic cell features.

The process of generating a decision tree can be automated by **machine learning**: an algorithm finds the optimal decision tree.



Generation of a decision tree



Viability decision tree

This can be further improved by using a machine learning known as **Random Forest Tree**. It creates several decision trees (algorithms) for classification from the same dataset and have the algorithms voting. Random forests are a way of averaging multiple deep decision trees, trained on different parts of the same dataset, with the goal of reducing the variance, thus improving the outcome of the classification

Based on QPI results, an **optical fingerprint specific for any cell type** can be created. As a consequence, it is possible to extract a specific fingerprint for a non-infected cell and another specific fingerprint for a cell infected by the BEVS. First, the system has to be trained using two data sets, one population made of non-infected cells only (typically at the beginning of the cell culture process) and one population made of infected cells only. Based on Random Forest Tree, it has been possible to design a detection algorithm that is capable of identifying cells that are infected.

Conclusions

- A limited dataset allowed us to design and train a detection system that can extract a specific cellular fingerprint pre- and post-infection, with a a false positive rate of 8.2% and a false negative rate of 7.3% were obtained
- This specific detection system allows in-line, continuous monitoring of an infection process when using Sf9 cells and BEVS

Future Developments

- The same principle of generating a fingerprint that is specific to a cell state could be used to determine in which part of the **cell cycle** a cell is.
- The availability of full data, per cell, for the whole experiment also allows **the use of the automated QPI 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 automated QPI system developed by OVIZIO (iLine F) can be **linked with the bioreactor controller**, allowing the control of the feeding strategy or the harvest time after viral infection, based on the cell density, the percentage of infected cells and the overall viability of the culture.

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