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In-situ microscopy: Realtime in-line monitoring of cell populations in bioreactors

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Concept

For monitoring bioprocesses microscopic methods are widely used. A sample is taken off the reactor and direct cultivation parameters as cell number and viability are determined offline. Using an in-situ microscope the cultivation process can be monitored directly within the bioreactor. A built-in image analysis allows to analyse in-situ acquired images in real-time and to determine process parameters like cell number in-line.
The in-situ microscope consists of the in-situ microscope hardware, the microcontroller, a compact PC with a frame grabber, an image analysis software and a control software for the microscope itself (Figure 1). In combination with the frame grabber and a progressive scan CCD-camera the in-situ microscope represents the image acquisition unit. The frame grabber digitizes the analog signal from the CCD-camera and the digital data can be evaluated by means of image analysis.

Figure 1:The in-situ microscope system

The in-situ microscope is based on standard brightfield transmitted light technology. To acquire images in-situ the microscope fits into a bioreactor's standard 25 mm side port. To sterilise the microscope body the interior steel tube with the objective and the other optical parts can be removed and reinserted without compromising sterility.

It is possible to retract the sampling zone of the in-situ microscope into a cleaning chamber. The principle is similar to the Intrac System by Mettler Toledo. Furthermore, the retractable probe housing allows to maintain and exchange the optical parts during the cultivation process.The illumination/condenser unit is located in the bottom part of the microscope and guarantees a homogenous illumination of the image. Figure 2 shows two images of mammalian cells acquired in-situ at different magnifications.

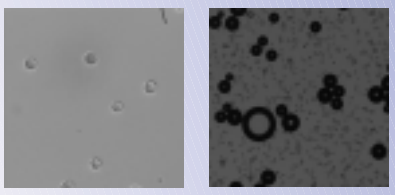

Figure 2: In-situ images of CHO cells, magnification 400fold (left), 80fold (right).The black rings represent air bubbles

The sampling zone

The images are acquired in a new flow-through sampling zone, see Figure 3. The distance between cover slip and objective slide, i.e. the height of the sampling zone, is set by a stepper motor attached to the outer tube. With the second stepper motor the inner tube with the objective can be adjusted for focussing purposes. A software autofocus system is available.

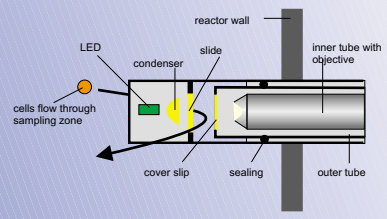

Figure 3: Flow-through sampling zone of the in-situ microscope

Image analysis

Figure 4 shows the image analysis algorithm for yeast cells and the integration into process control.
First, the areas with maximum brightness in the defocussed image, representing cells, are being amplified by filter operations. With a Maximum Likelihood method the optimal threshold of the image is determined automatically. With this threshold the image is segmented into two regions: background and regions possibly representing cells. In a next step neighboured pixels with equal grey value are joined to global regions (region growing). These regions are being labelled and then analysed according size and morphology respectively. The resulting image is compared to the original image and as a result the numbers of single cells, double cells and cell clusters are calculated.
These parameters are used to estimate cultivation parameters online, e.g. cellular dry weight or cell size distribution. These parameters again can be used to optimise bioprocess parameters as, for example, the substrate concentration.

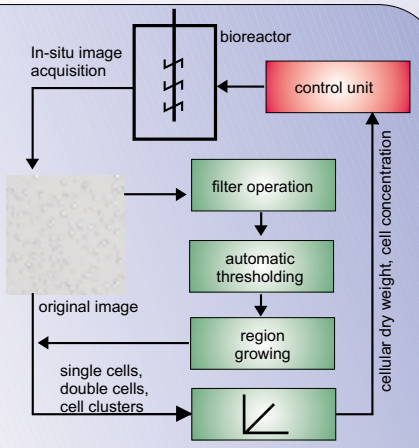

Figure 4: Integration of yeast cell image analysis into the process control

Results

The concentration of yeast cells (*S. cerevisiae*) in a 3 L bioreactor was increased in defined steps. Figure 5 shows the linear dependency between the results of the in-situ microscope (cells per image) and the cell number determined with a hemacytometer chamber. Furthermore, the in-situ microscope was used to monitor a CHO perfusion cultivation in a 5 L reactor. The number of cells determined with the in-situ microscope and with the Cedex system (Innovatis AG, Bielefeld, Germany) as a reference method correlate well.

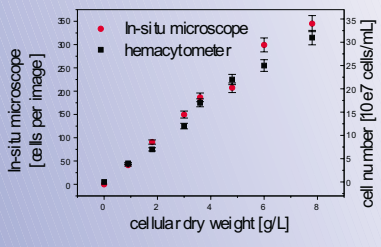

Figure 5: Linear dependency of hemacytometer offline and in-situ microscope online counting

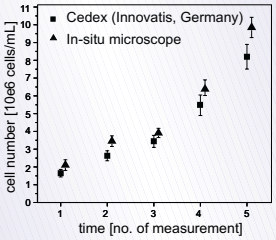

Figure 6: Comparison of cell counting results during mammalian cell cultivation

Conclusion

The results obtained with the in-situ microscope correlate well with established cell counting methods as for example hemacytometer or Cedex system. Using the in-situ microscope, it is no longer necessary to take samples, a bypass is not needed. Subjective errors by manual counting are excluded. In combination with a well adapted image analysis it is possible to determine most of the interesting cell parameters in-line. A complete automation of the bioprocess is possible.
Using mathematical methods as, for example, the continous discrete extended Kalman-Filter (EKF) in combination with the in-situ microscope measurements, bioprocess parameters as substrate concentration can be optimised.
Long term runs demonstrated the ability of the system to guarantee sterility and robustness for perfusion cultivations. Because of its modular design the in-situ microscope is user friendly and easy to maintain.