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Online Monitoring of Microcarrier-Based Adherent Mammalian Cell Cultivations with In-situ Microscopy

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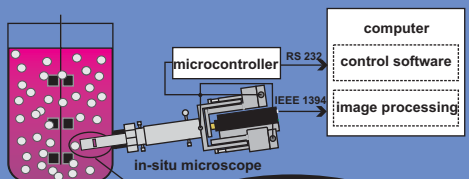
Introduction

Taking offline samples is time-consuming and risk culture contaminations during sampling. Culture inline monitoring and online data analysis helps to reduce manual samplings to a minimum and thus reduces the risk of contaminations. In this context in-situ microscopy has proven to be a reliable analysis method for different process parameters in cultivations of yeasts and mammalian cells. This has already been described in the literature [1-4].
To achieve high density cultures of anchorage-dependent cells the use of microcarriers is a common procedure. For adherent cells process parameters like plating efficiency (PE) and the average level of colonisation (LOC) on the available microcarrier surface are most important for process management, harvest or in this case for the inoculation time of virus. To increase yields such parameters are measured ideally online.

In-situ Microscope Implementation

ISM III-XTF Features:
- Easy installation and handling
- Mounting via 25 mm side port
- High resolution images (1280x960)
- SIP and CIP (retract mechanism)
- Adjustable flow through sampling zone
- Long term stable
- Bright field and dark field optics



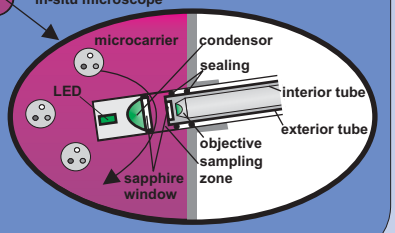


Fig. 1: In-situ microscope III-XTF.

Cultivation Monitoring

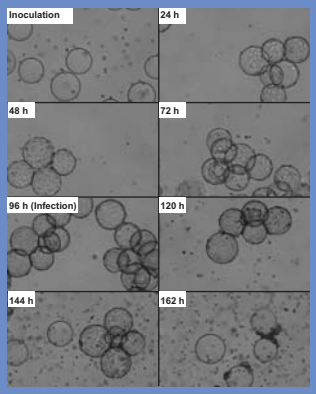
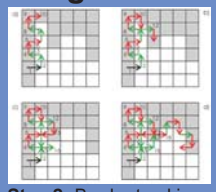


Fig. 2: Cell growth of adherent canine MDCK cells (permissive for influenza virus) on microcarriers monitored online with an in-situ microscope (4-fold magnification). The cell attachment in combination with morphological changes can be recognised. The PE is nearly 100%.

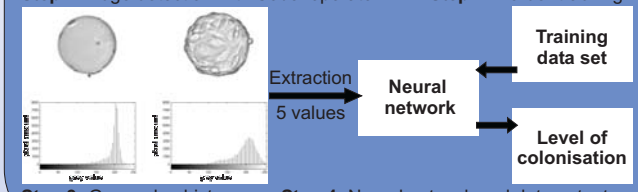
The cells were infected with virus after 96 h cultivation time. After 120 h the cytopathic effect is considerably detectable. In the course of infection more and more dead cells detach from the microcarrier surface (144 h) until most microcarriers are completely uncolonised (162 h).

Microcarrier Image Analysis Algorithm

Step 1: Edge detection with Sobel-operator.



Step 2: Border tracking.



Step 3: Grey value histogram. **Step 4:** Neural network and data output.

Results

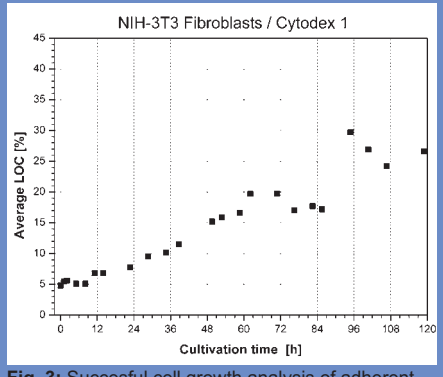


Fig. 3: Succesful cell growth analysis of adherent mouse NIH-3T3 cells on Cytodex 1 microcarriers.

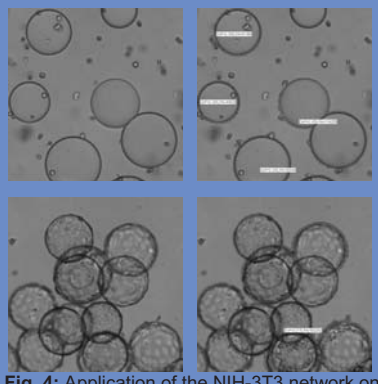


Fig. 4: Application of the NIH-3T3 network on inline images of MDCK cells.

Conclusion

In a first attempt it was tried to apply the successful network of mouse fibroblast cells on MDCK cells. Although the same type of microcarriers were used the cells on the surface appeared slightly different so that the network failed. Beside that the image analysis algorithm is not suitable for this task due to the strong affinity of the MDCK cells to form extremly large agglomerates shortly after inoculation. The algorithm is suitable to analyse single microcarriers or small agglomerates (3 - 4 microcarriers) in a 2D layer. For this application the algorithm must be advanced to enable the analysis of microcarriers in three-dimensional layers according to suspension cell analysis algorithms already published [5-6].

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[4] Wei et al., Biotechnol Lett 29, 373-378, 2007.
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