Observation and Analysis of Lab Scale Microcarrier Cultivation by In-situ Microscopy with Image Processing Tools

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Introduction

The monitoring of biomass for concentration determination or morphological studies is of main interest in modern bioprocess analysis. Different methods are available to monitor biomass concentration but no system is able to analyse concentration and morphology simultanously online. In-situ microscopy offers the possibility for both. Different applications for yeast-, BHK and CHO-cell cultivations are already described.

Microcarriers are widely used in bioprocesses and are well suited to achieve high density cell cultures. Adherent cells grow on the particle's surface so that the cells can be cultured in a volume instead of a two-dimensional surface as monolayers. The accessible cell densities in culture volume are up to 20 times higher compared to a monolayer bottle.

In this work we present the possibility to analyse lab-scale microcarrier cultivation with in-situ microscopy. Our aim for the future is to develop an image processing routine to monitor the cell growth on the microcarriers online. Therefore we are working with a model cell line (NIH-3T3) cultivated on Cytodex™ 1. This type of microcarriers has been selected because of its transparent properties which make them ideal for in-situ microscopy. Cytodex™ 1 microcarriers attached with adherent cells can be easily distinguished from non-colonised micocarriers.

The first step on the way to an automated analysis tool is the grey value distribution's interpretion of the images taken by the in-situ microscope during a cultivation's course. The transparency of a microcarrier decreases whereas the average of the grey value distribution rises.

Cultivations (Fig. 6)are performed in a self-built 5 L steel tank reactor with an adjusted 25 mm port for the ISM. The chosen media consists of 50% DMEM and 50% RPMI with 3,25 g/L glucose and 10% NCS. The microcarrier concentration is 2 g/L. The ISM is equipped with 4fold optical magnification (total magnification 450fold) and a Sony CCD-Camera.

Concept of In-situ Microscopy

Features:
- Brightfield transmitted light microscopy (LED illum.)
- High resolution Fire-Wire CCD-camera (1280 x960)
- Finity corrected objectives
- 4fold, 10fold and 20fold optical magnification
- Full autoclaveable
- Cleaning of sampling zone by retract system

Measured parameters:
- Cell concentrarion
- Cell size distribution
- Cell volume (biomass)
- Differentiation (single cells, double cells, clusters)

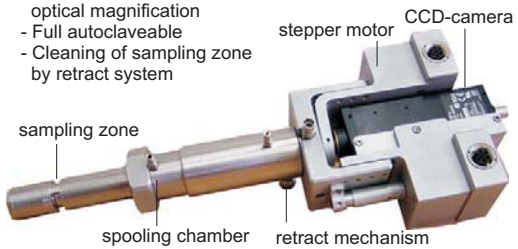

Figure 1: In-situ microscope system - Type III-XTF.

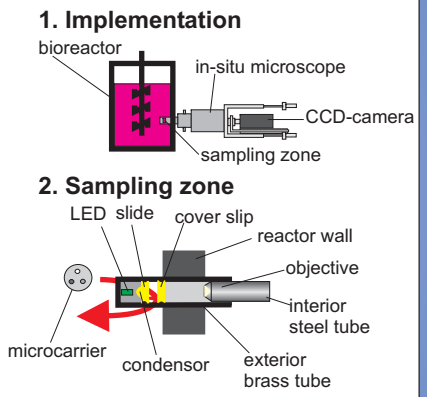

Figure 2: Flow-through sampling zone.

Applications

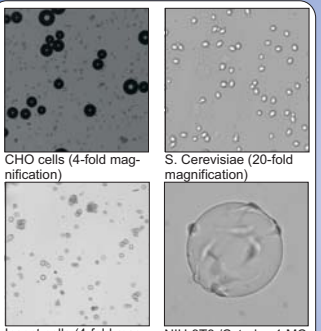

Figure 3: Inline cell images.

In-situ Control & Analysis

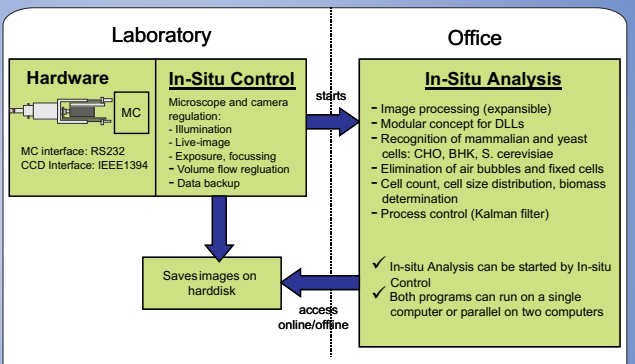

Figure 4: Software concept for in-situ microscopy.

Results

Example: image processing with YeastCellCounter

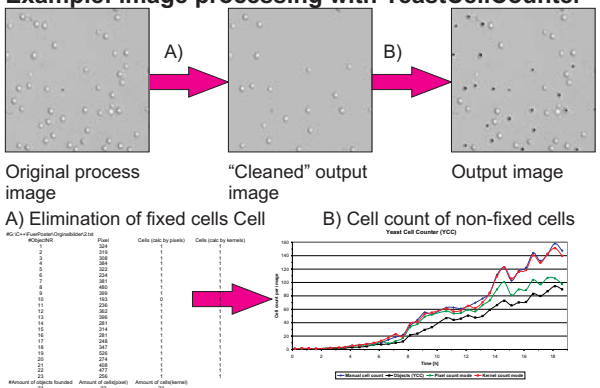

Figure 5: Cell count results of a yeast batch cultivation.

Inline monitoring of NIH-3T3/ Cytodex™ 1 microcarriers

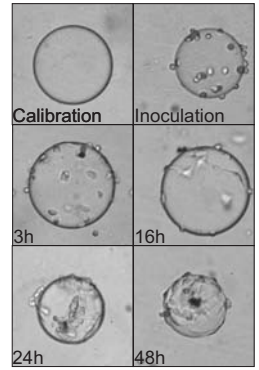

Figure 6: Batch cultivation of NIH-3T3.

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