

Synchronization of eukaryotic cells for Systems Biology Research

O. Platas Barradas*, U. Jandt, S. Schubert, R. Pörtner and A.-P. Zeng.

Abstract

The synchronization of eukaryotic cells implies the enrichment of cells within a determined phase of the cell cycle. After synchronization, further synchronous cell growth is a main requirement for systems biology studies, since synchronized cell populations allow scientists to study and predict the dynamic of complex cell-cycle-depending pathways. Our work focus on the study of physical synchronization methods for a human production cell line (AGE1.hn). These methods are being tested for their feasibility of yielding a high number of synchronous cells, which should further divide synchronously and unperturbed. After characterization of the methods, a reliable synchronization procedure will be established at our institute, which allow for a deeper study of the „omics“ of eukaryotic cell lines. The number of cells needed for our studies systems can be often very as high as $1 \cdot 10^8$ cells per sample, which can be hardly obtained from single batch cultures. For this a dialysis bioreactor will be employed, which allow for unperturbed cell division for many divisions cycles before any indication of limitation or inhibition appears. Our results point to the feasibility for cell synchronization. The use of the dialysis bioreactor will provide information about the number of synchronous divisions after method employment.

1) Temperature Reduction

Cell growth can be arrested by means of a reduction of temperature during culture, which allows for enrichment of cell populations within the G1 and early S-phase. Cell synchronization was induced by temperature reduction during shake flask and bioreactor culture.

Pre-experiments showed an increase of up to 80% of S-Phase DNA-content during culture at 30°C. Further temperature reduction was needed during repeated batch cultivation with temperature cycles for cell growth arrest. Viability decrease after temperature resumption (37°C) might be due to apoptosis.

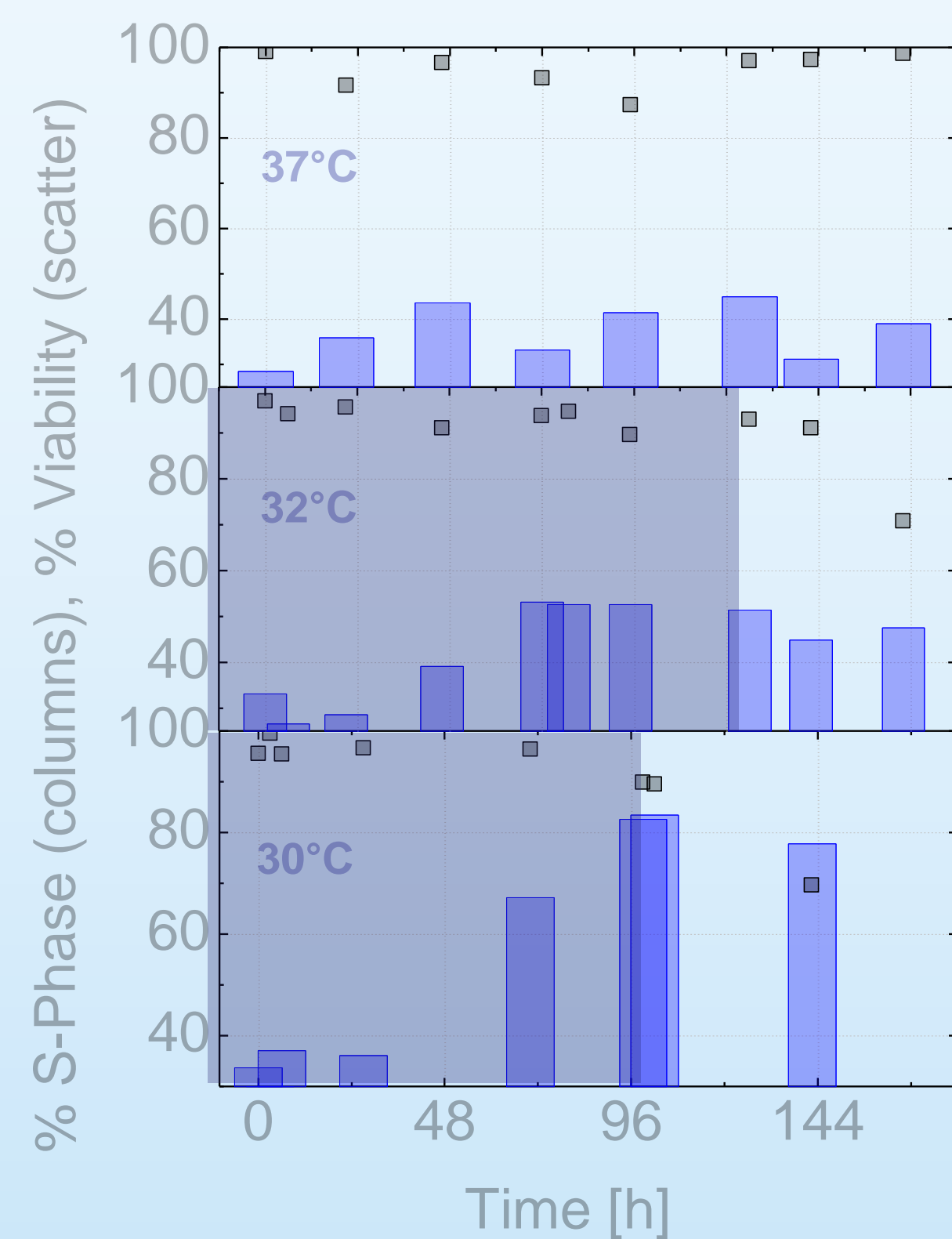


Figure 1: Fraction of cells in S-Phase during cultivation at different temperatures.
■ Reduced temperature

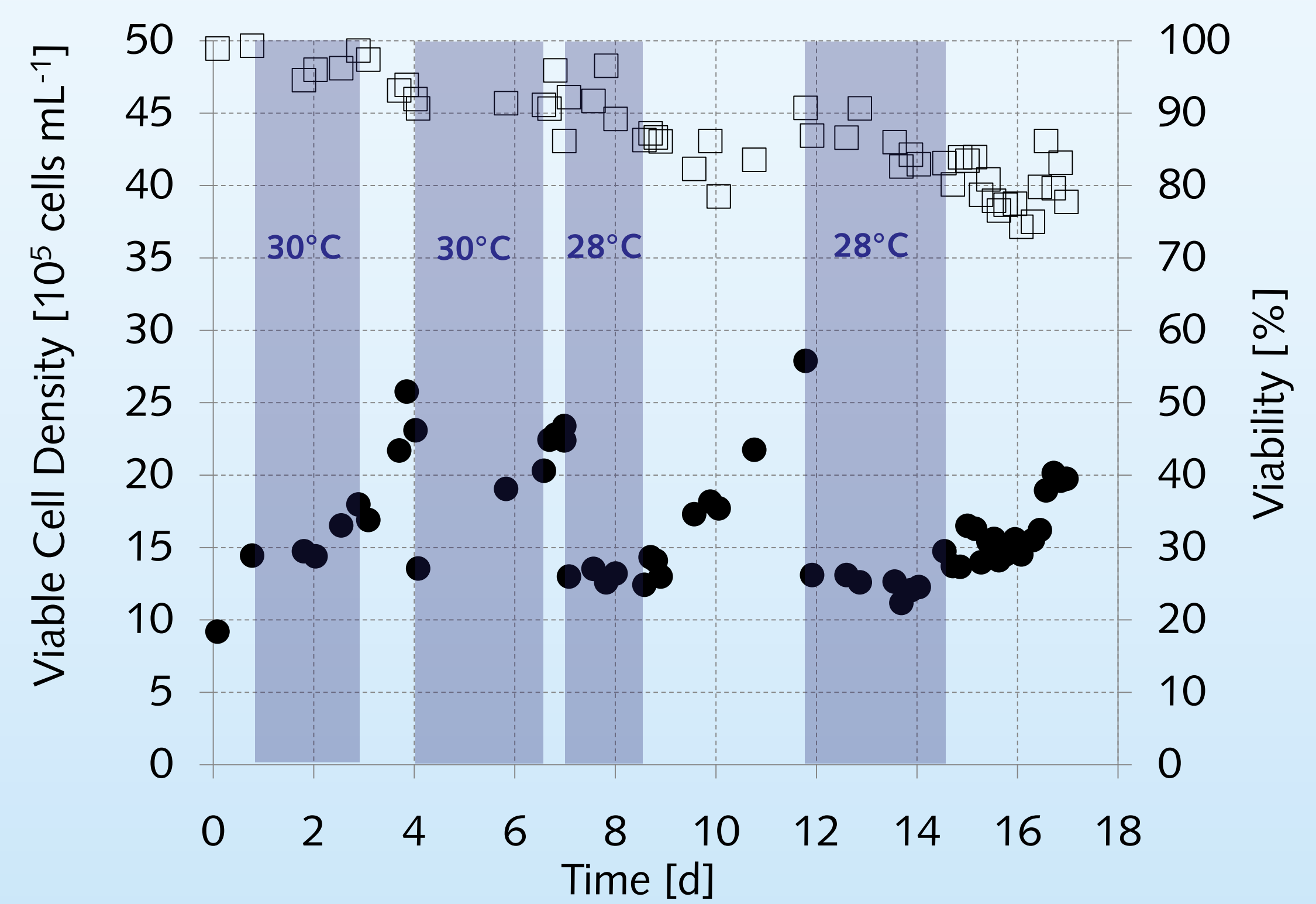


Figure 2: Repeated batch experiment for induction of synchronisation by means of temperature cycles. As cells showed clear growth during the second 30°C cycle, temperature was reduced at 28°C. Samples were taken for cell cycle analysis (results not shown).

2) Gradient centrifugation

Cells can be separated by centrifugation according to their size in solutions with higher density than water. Sucrose/Ficoll gradients can be used for this purpose. This method has the advantage of being simple and not expensive.

Using a combination of Sucrose and Ficoll, high density gradients can be obtained without relevant increase of the osmolarity.

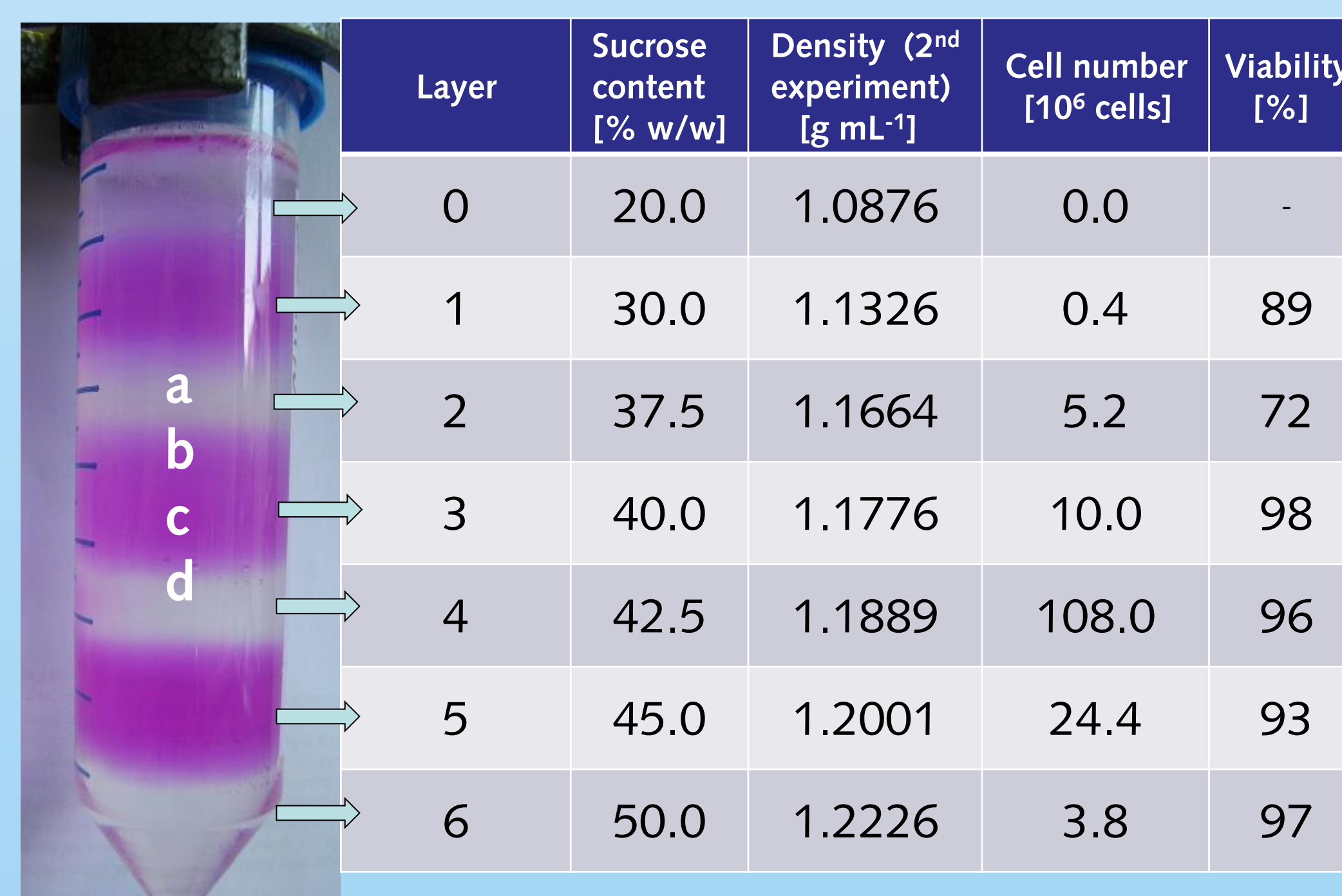


Figure 3: Sucrose gradient coloured with phenol rot for layer identification. The density indicated for each layer was measured at a temperature of 25°C. Relevant samples were taken as indicated by the letters (a-d). Cell count and viability are displayed in the table.

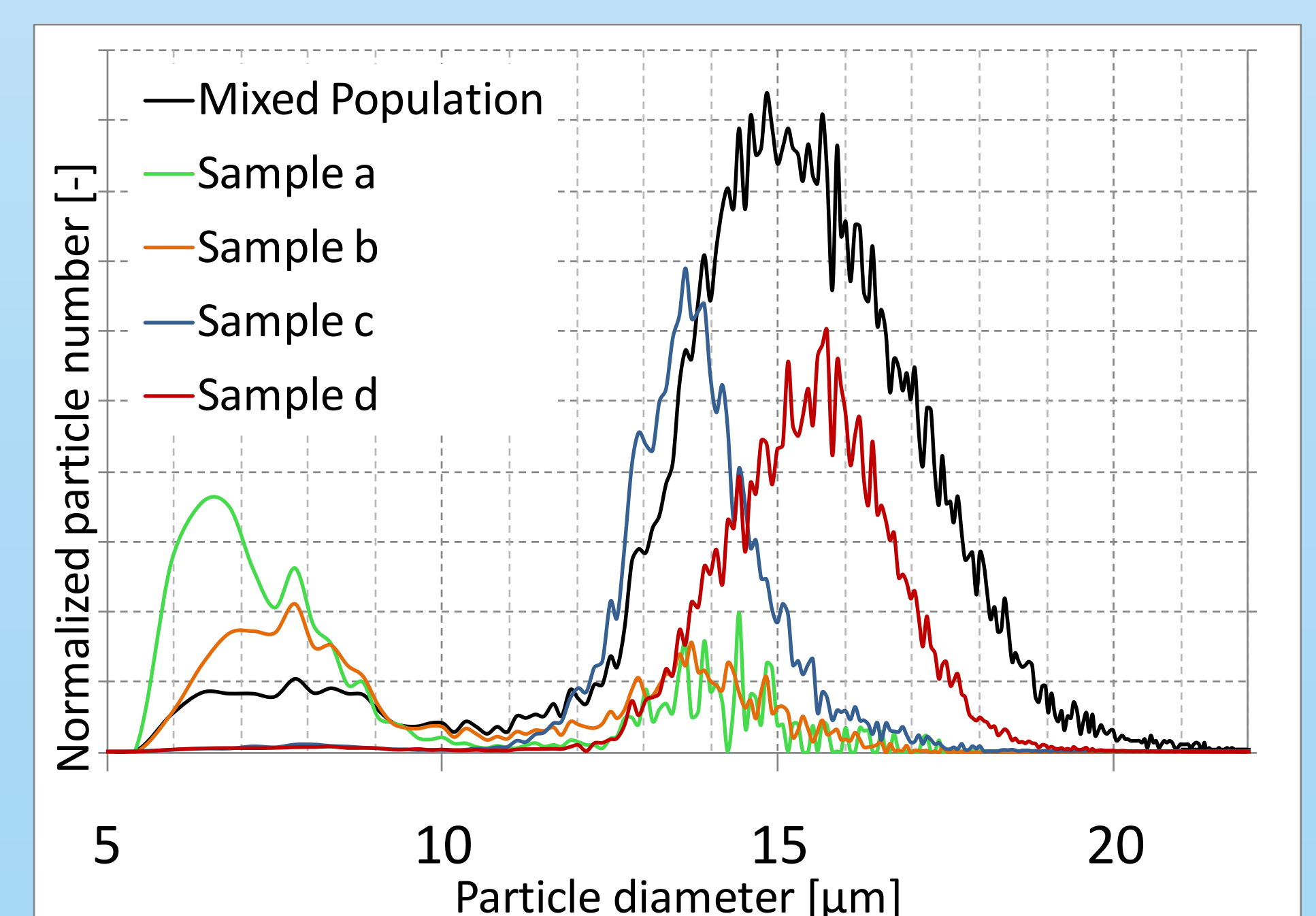


Figure 4: Normalized particle count after sampling (a-d). Count was performed with a Counter Coulter Z2 (Beckman Coulter)

3) Countercurrent centrifugal elutriation

Sedimentation of cells in a centrifugal field is arrested by a fluid in countercurrent flow inside a separation chamber. After increasing the fluid velocity, cells are eluted from the separation chamber according to their mass.

This method has shown a high yield of cells enriched in different phases of the cell cycle (table 1). Further culture of the elutriated fractions was possible without noticeable perturbation of cell growth (figure 6).

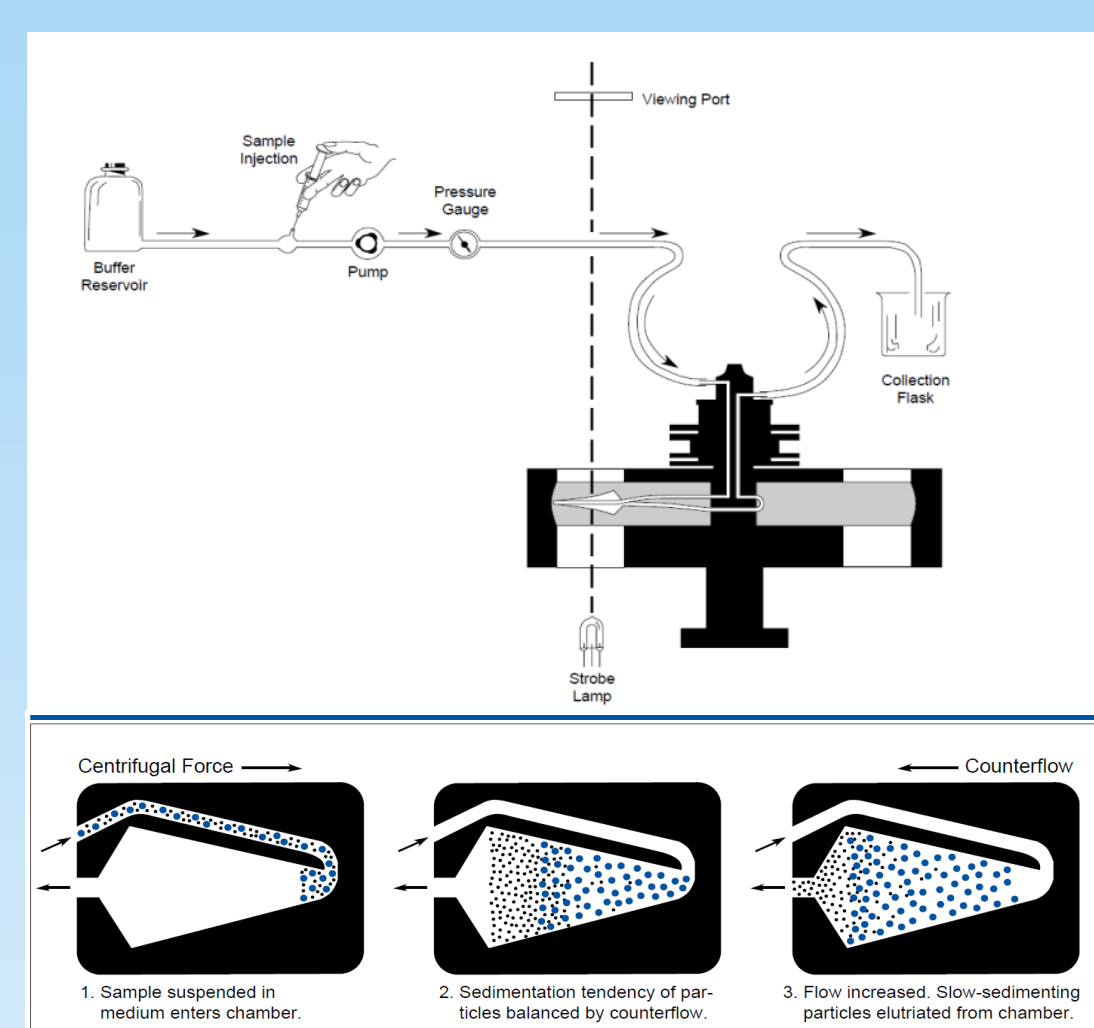
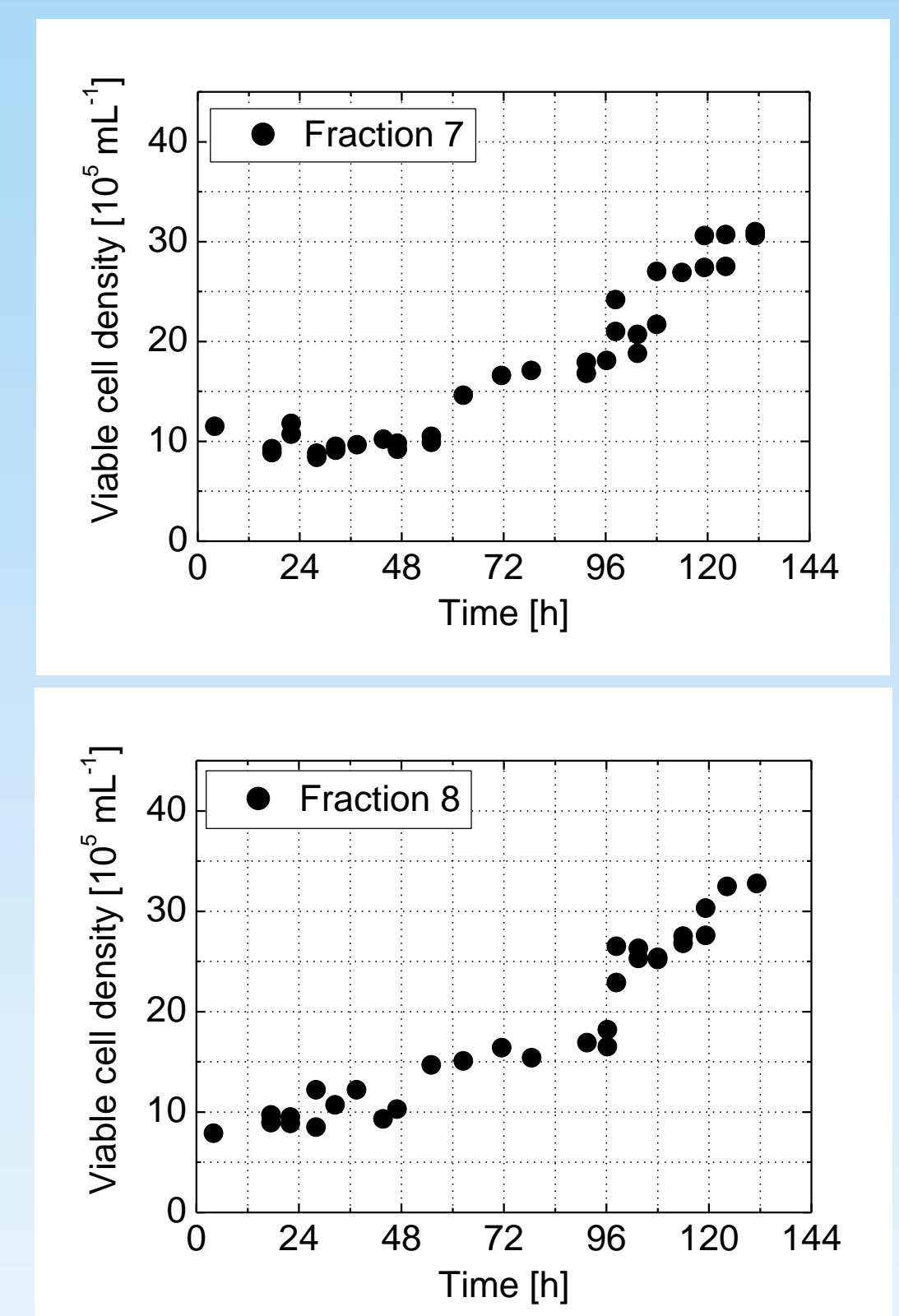


Figure 5: Principle of the elutriator system: (a) Cells are pumped in sterile conditions into a separation chamber which rotates at a defined velocity. By increasing the flow rate of buffer solution, cells are eluted out of the system and can be collected for further work (Source: Beckman Coulter).

Table 1: Flow cytometry analysis of the elutriated fractions. The first column shows the cell cycle profile of a heterogeneous cell population. By means of this principle, the fraction of cells in a determined phase can be increased up to 30% (see red numbers).

	Mix AGE 1	Elu1	Elu2	Elu3	Elu4	Elu5	Elu6	Elu7	Elu8	Elu9
sub G1	1,5	17,3	0,8	0,8	0,5	0,9	1	0,5	0,6	3,1
G0/G1	61,6	50,9	79	76,4	63,4	60,8	50,9	25,3	5,9	15,7
S	17,8	4,6	9,9	11,3	22,2	25,2	32,9	47,6	20,8	17,4
G2/M	14,2	9,4	5,8	7,1	7,2	7,2	10	21,2	52,6	35,6

Figure 6 (right): 6 day culture of two elutriated fractions. Stepwise growth can be observed, which is an indicator for synchrony in culture.



* Oscar Platas Barradas (corresponding Author), e-mail: o_platas@tuhh.de

¹ Hamburg University of Technology. Bioprocess and Biosystems Engineering Denickestr. 15, D-21073 Hamburg, Germany. www.tuhh.de/ibb

Funding:

This work is a part of SysLogics: Systems biology of cell culture for biologics, a project founded by the German Ministry for Education and Research (BMBF).