Analysis of Floc Systems with Inline Imaging Methods for the Understanding and Process Optimisation of Shear Stress in Cell Systems

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The use of biopharmaceuticals experiences a significant growth. The use of microorganisms for the production of active pharmaceutical ingredients as well as cell-based therapies have become increasingly important as global, annual revenues of approximately 25 billion US$ indicate. The production of biopharmaceuticals, known as bioprocess, involves a wide range of techniques, apparatuses and process conditions. Proper mixing is necessary in many of those applications but mixing is usually introducing shear stress. Cell damage due to shear stress is decreasing the production rate and efficiency of bio processes significantly. The understanding and minimization of cell damage with optimised mixing conditions is necessary for further process optimisations. Floccular systems, have been used as model systems to study the shear stress in a wave-mixed single-use bioreactor [1].

Material and Methods

All experiments were conducted in a CELL-tainer® 20L under the variation of the following parameters: working volume V, rocking rate k, rocking angle φ. Particularly in sensitive and instable systems, the dynamic quantitative size measurement of particles is a major challenge. In contrast to sampling, which is time-consuming and involves the danger of adulteration, an in-situ working analysis method with high spatial and temporal resolution is selected to measure the floc size distribution (FSD). [2, 3] The photo-optical SOPAT measuring technique for particle sizing is capable of acquiring raw data (two-dimensional images) of the dispersed phase (here: solid flocs) during the process and measure the sizes and shape by means of automated image processing followed by an analysis, see Fig. 1 (www.sopat.eu).

Results and Discussion

The experiments showed clearly that the used floccular system in combination with in-situ photo-optical size measurement lead to a better description and understanding of shear stress in the investigated wave-mixed bioreactor. A decrease of floc sizes over time down to a stable mean size as well as a decrease for higher power input were observed. Due to the high sensitivity of the model and measurement system, a precise differentiation of operating points was possible dependent only on the reproducibility of the setup.

Whereas averaged diameters decreased over time, a more detailed insight by evaluation of FSDs in their entirety is obtainable. Thanks to the high spatial resolution, not only an average diameter of the flocs was measurable but precise and reliable shape analysis was performed. Such dynamic and in-situ shape information (e.g. sphericity) reveal deeper insights to the kinetics of flocculation and floc destruction. Additionally, the measurement technique could not only detect the required FSD, but also was able to distinguish between different dispersed phases, for example disturbing bubbles. As the results are available inline and in real-time, a closed control loop can be established.

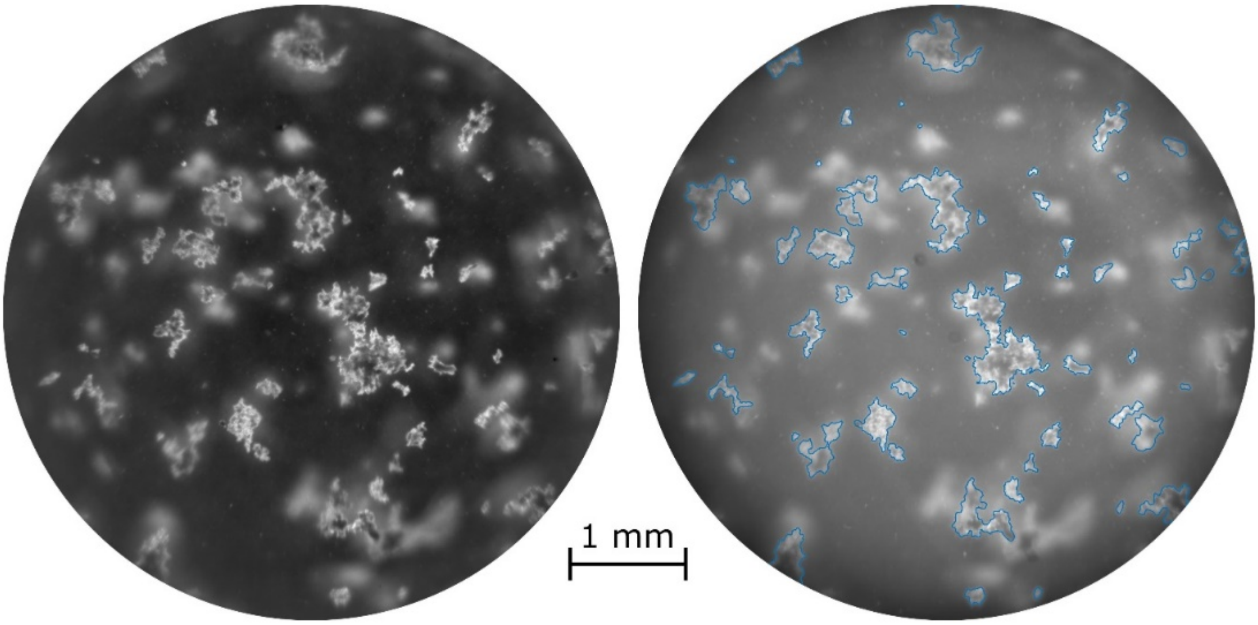

Fig. 1: Photo-Optical Measuring Method for Particle Size Distributions, Left: Original Image after Image Processing and Right Original Image with Detection Results (Blue Contours)

References

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