doi 10.18699/bgrs2024-9.2-26

Shedding light on cell proliferation: cell culture monitoring using light scattering

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Key words: cell proliferation; label-free real-time cell analysis

Motivation and Aim: Investigations of cell growth and division enlighten the underlying mechanisms of cellular proliferation, factors that regulate cell cycle progression, and the responses of cells to various stimuli. These studies play a vital role in various scientific fields including cancer research and pharmacology. By now, various techniques and assays to access cell proliferation exist such as cell counting using hemocytometer/automated cell counters, colorimetric assays (MTT), fluorescence (CyQUANT)/ bioluminescence assays (luciferase enzymatic reaction), electric impedance measurements (xCELLigence), etc. However, the development of cost-effective, non-invasive and easy-to-perform methods to trace cell proliferation is of high need and importance. Herein we focus on the development and validation of the device monitoring dynamics of both adherent and suspension cell cultures using laser light scattering.

Methods and Algorithms: In our work we performed numerous modifications of the device developed in our laboratory before[1]. The initial optical scheme of the device incorporates a 850 nm, 3 mW dot laser module and a set of photodiodes BPW34 organized in a row to be compatible with the width of T25 biological cell flask. As the laser beam passes through the flask at the distance of 1 mm above the flask bottom, the light scattered by the cells is detected by photodiodes allowing us to carry out non-invasive suspension cell monitoring.

However, common biological models utilized in the laboratory practice include both adherent and suspension cell lines. Consequently, we decided to extend the optical part of the device with the second laser of 660 nm, 5 mW illuminating the sample flask bottom to access the dynamics of adherent cell layer formation as well. In the pilot experiment, we investigated the process of time-dependent sedimentation of *E. coli* cells in the course of 3 days. Next, we analyzed HEK293 adherent cell layer formation within the time range of 4 days.

To further validate our method, we decided to (i) determine the dynamic range of our device for cells in suspension using different concentrations of a number of suspension cell lines as a model system (ii) use our device to access the proliferation of several adherent cell lines at the different seeding density in parallel (iii) perform a fluorescent staining of the cell samples in (ii) to verify the data obtained with our device.

Inspired by recent advances in fingerprint recognition by means of frustrated total internal reflection, we proceeded with more sophisticated optical design for adherent cell layer tracking. Currently, we are modifying the instrument with the prism embedded in the device body so that it gets in a tight contact with the plastic bottom of the sample flask once the flask is placed to be measured. As the light enters the prism, it gets

redirected and enters the bottom of the cell flask in a total-internal-reflection mode. The "trapped" light can interact with adherent cells resulting in the increased scattered light intensity detected by photodiode sensors.

Results: The results of the experiments with bacterial cell culture sedimentation showed the expected patterns in light-scattering intensity change over the time for both lasers. We observed a decrease of the scattering signal for upper laser beam and an increase of the signal for lower laser beam due to the formation of sedimented cell layer. Subsequently, we saw the same trends for the adherent cell culture model (HEK293 cells). Next, we performed experiments to determine the dynamic range of the instrument for the suspension cell cultures. Also, we tested the device for various adherent cell cultures. The experimental data allow us to estimate the reproducibility and accuracy of the device measurements.

Conclusion: We have developed a brand-new optical device allowing one to monitor cell proliferation for both adherent and suspension cell cultures in a commonly used T25 flask. When compared to other existing techniques, it has several advantages as it allows to perform non-invasive, cost-effective, label-free cell proliferation studies for both adherent and suspension cell populations simultaneously. The device is intuitive to operate and can be easily transported as it is compact and light (0.2 kg). Also, the important figures of merit of the instrument are continuous readout and a high measurement speed (1 s). Currently, device utilization is limited to single sample measurements. Therefore, we are working on the possible solutions to this challenge. The evolving field of cell proliferation studies includes investigations of cell metastatic transformations[2]. From this perspective, it might be advantageous to use an approach based on total internal reflection to differentiate between adherent and lying on the bottom cell populations. We shall therefore continue optimizing the methods described above to hopefully lay a foundation for novel cell research technologies.

Funding: The study was supported by the Ministry of Science and Higher Education of the Russian Federation (project FSUS-2020-0039).

Acknowledgments: We express our deepest gratitude to Petr Laktionov (Laboratory of Epigenetics, NSU) for providing us with the biological samples, for our fruitful scientific discussions of the device development strategies and his word of advice. Likewise, we also thank

Sergey Kulemzin (Institute of Molecular and Cellular Biology SB RAS) for his valuable and constructive input. Furthermore, we thank the entire team of the Laboratory of Optics and Dynamics of Biological Systems for the support, help and encouraging research environment.

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