Wireless monitoring of cell cultures based on light scattering: A novel optical scheme and portable prototype

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Abstract: Cell cultures are widely used in scientific research, biomedicine and industry. When culturing, it is important to maintain certain conditions, including the concentration of cells. Monitoring of the culture growth and cell counting is an urgent task for the optimization of technological processes. Most existing methods require sampling from a culture flask. This procedure is time-consuming and associated with the risks of contamination. We present a device able to monitor the growth of cells number in a suspension non-invasively. The device uses a laser beam that pass through the culture flask and measures the intensity of scattered light as a function of coordinate along the beam. This optical scheme allows one to obtain accurate results for both high- and low-scattering samples. We constructed the wireless portable prototype for monitoring of cell culture growth directly in the incubator and demonstrated the applicability of the device for Jurkat cells and *e.coli* bacteria.

1. Introduction

Cell cultures are widely used in today's industry and research. For instance, they are used to produce hormones, enzymes, and other complex organic substances (1). The cultivation of bacteria is significant for a vast block of the food industry (2). Cells are an irreplaceable laboratory material in biomedical research, and the cultivation of cancer cells has completely revolutionized the field (3). Biotechnology cannot be imagined without the use of cell cultures as well (4,5).

It is substantial to maintain certain conditions during cultivation, including the concentration of cells (6). For all cells, and especially for bacteria, growth phases can be distinguished. A lag phase is followed by period of active division (log phase) and then by a stationary phase, during which the number of cells remains approximately constant. It is important to differentiate between growth phases for different applications, and for the prevention of culture death.

Most widespread devices for cell counting use special microscope slides or Coulter principle and require sampling of the material. As cells are cultured under sterile conditions and often inside specialized equipment (for instance, CO_2 incubators), frequent cell counting is problematic from practical point of view because: 1) the operator should use special uniform (at least gloves and lab coat) and disinfectants; 2) the risk of contamination increases, including the contamination of culture and possible of the operator; 3) sampling leads to a change of a volume and possibly other parameters of the cell environment; 4) sampling and measurements themselves require consumables; 5) this type of measurement is time-consuming not only due to the sample handling but also due to the need to use the laminar flow cabinet which requires several minutes to start.

There are several devices that do not require sampling. The first approach is based on the measurement of electrical impedance (7,8). The second approach uses miniaturized microscope (9). Both approaches can be used for real-time monitoring of cell culture growth, and there are corresponding devices on the market, but they are designed for the adherent cultures only. On the other hand, many important cultures are suspension cells, e.g. lymphocytes used for CAR-T therapy and most bacteria used in biotechnologies.

In this paper, we present a device that is able to monitor the growth of a suspension cell culture non-invasively. The device uses a laser beam passing through the culture flask and measures the intensity of scattered light as a function of coordinate along the beam. This optical scheme allows one to obtain accurate results for both high- and low-scattering samples. We have constructed the wireless portable prototype for monitoring of cell culture growth directly in the incubator and demonstrate the applicability of the device for Jurkat cells and *e.coli* bacteria.

2. Results

2.1. Device Implementation

The working principle of the device is as follows. Laser beam passes through the culture medium, and the scattered light is measured by eight sensors which are located along the beam as shown in Fig. 1A. Each sensor is sensitive to the particular segment of the laser beam, therefore it is possible to quantify the attenuation of the beam in the medium. Thus, this optical scheme incorporates nephelometry, which is known to be the most sensitive approach for the detection of particles, and turbidimetry, which is widely used for highly scattering and/or absorbing samples.

Figure 1B shows major components of the device. The optical part consists of the 850 nm, 3 mW dot laser module and 3D-printed cover with 8 square holes which match the size and positions of photodiodes. Photodiodes (BPW34) are located in a row at specially designed board. Electronics of the device consists of two ADC modules (ADS1115, 16bit ADC + up to 16x programmable gain amplifier), Arduino NANO microcontroller and the Bluetooth module. The prototype is also equipped with servo motor used as a shaker for the culture flask and 9V power supply. Figure 1C show the photo of the prototype which was used for pilot studies.

The laser wavelength (850 nm) was selected because: (1) most culture media are transparent, even those containing pH-sensitive dyes; (2) most cells are non-absorbing, with the exception of red blood cells; (3) the silicon photodiodes have the sensitivity maximum nearby.

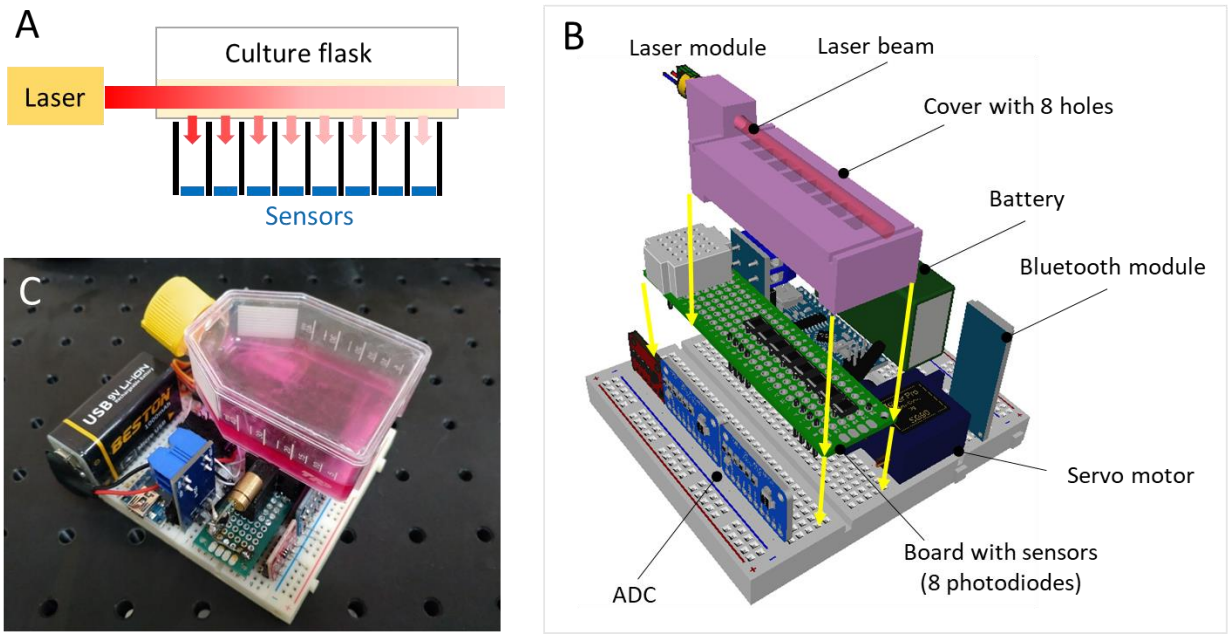

Figure 1. A. Measurement principle. Laser beam goes through the culture flask and is scattered by the medium containing cells. The intensity of scattered light is measured by eight sensors, each having field of view limited to a certain segment of the laser beam. B. 3D model of the device showing its major components. C. Hardware implementation of the device.

2.2. Theory

Light scattering by particles with sizes comparable to the wavelength is wave-optical phenomenon. For instance, Lorenz-Mie solution describes scattering of plane incident wave by individual spherical particles, as well as two- and multi-layered spheres. This solution allows relatively fast calculations, and many computer codes are available for this purpose. Theories and codes for particles with complex shape also exist, although are more complicated. Generally, for almost any given optical model it is now possible to solve the light-scattering problem and obtain its characteristics, such as the extinction, absorption and scattering cross-sections C_{ext}, C_{abs}, C_{sca}, the asymmetry parameter g and generally the scattering intensity as a function of scattering direction.

In the case of single (independent) scattering and non-absorbing particles, the attenuation of light by the sample with thickness x containing particles with a concentration n is given by the Beer's law:

$$I_{\parallel}(x) = I_0 e^{-nC_{\text{sca}}x} \tag{1}$$

where I_0 is the intensity of the incident beam and $I_{\parallel}$ is the intensity of beam after passing the sample. However, in reality disperse media consist of many particles which may re-scatter light from each other, the phenomenon known as multiple scattering. It significantly complicates the theoretical description of the system, because each particle configuration technically implies separate light-

scattering problem. The theoretical description of the averaged measurable characteristics is possible using the radiative transfer equation (RTE). Numerical codes for the solution of RTE are available, for instance, those based on Monte-Carlo simulations of discrete rays of light.

In our experiments, the laser wavelength is selected so that the absorption is negligible and light is attenuated only due to scattering. Also the scattering is relatively weak, so that one can use the simplest approximation to the RTE called the "photon diffusion". The scattering by each particle can be decomposed to isotropic part with the weight $(1-g)$ and forward-directed part with the weight g. Accordingly, the beam attenuation coefficient is reduced:

$$I_{\parallel}(x) = I_0 e^{-nC_{\mathrm{sca}}(1-g)x}. \tag{2}$$

The intensity of light scattered perpendicular to the beam is given by

$$I_{\perp}(x) = \alpha \cdot n \cdot I_{\parallel}(x), \tag{3}$$

where α is the proportionality constant incorporating the sensitivity of the sensors, gain, scattering intensity near 90°, collection angle and the volume from where the scattered light approaches the sensor.

2.3. Experiment with polystyrene beads

To test the theoretical prediction (3), we performed measurements with 3.7 μm polystyrene beads (ThermoFisher Scientific, Cat. # C37253) as follows. First, we diluted the stock solution of beads 100-fold and measured the obtained sample with our device. The obtained data are plotted in Figure 2 as blue points and designated by C_0. After that we diluted the sample again 2-fold, measured the data and repeated this step 4 times. Experimental results are shown in the Figure 2 as points.

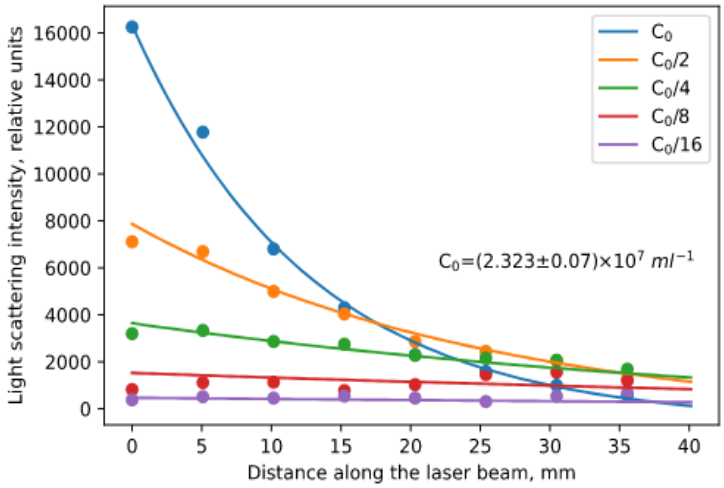

Figure 2. Experimental measurements (points) and theory (lines) for polystyrene beads in different concentrations. The first concentration C_0 and the proportionality coefficient are determined by fitting.

Next, we performed fitting of the experimental data using Eqs. (2) and (3). The unknown parameters are the concentration C_0 and the proportionality constant α, whereas the scattering cross-section C_{sca} and the asymmetry parameter g were calculated using Mie theory. In principle, one curve with profound exponential decay is enough for the determination of concentration, but in that case the precision would be much worse. Here we used the results of all experiments simultaneously. The results of fitting are shown in the figure 2 as lines, and the obtained concentration is $C_0 = (2.32 \pm 0.07) \times 10^7$ ml^{-1}. This value is higher than the concentration estimated from the manufacturer's datasheet (4% v/w corresponds to ~1.5×10^7 ml^{-1} after 100-fold dilution).

However, we can conclude that our simple approach gives reasonable results (up to a factor of 1.5), which is good enough for many applications. When monitoring cell cultures, there is no strictly defined optical model: cells are significantly different, have irregular shapes and change shape and volume during cultivation. Therefore, only the estimation of some effective concentration is possible.

2.4. Cell culture testing

Biological cells usually have the relative refractive index (RI) close to 1 (e.g., 1.01-1.05) and lower scattering intensity than polystyrene beads with comparable size. Side scattering (which is measured by our prototype) is several orders of magnitude lower than forward scattering, especially for large optically soft particles with homogeneous internal structure (10,11).

First, we tested if the signal is measurable for the culture of Jurkat cells. Jurkat cells are an immortalized line of human T lymphocyte cells widely used in scientific research. They have highly spherical shape with the diameter ranging from 10 to 16 μm (12) and large nucleus, with the RI in range 1.36–1.39 (relative RI 1.02–1.04) (13). Normally, Jurkat cells are maintained in concentration between 10^5 and 10^6 cells/ml. We measured the sample with high concentration (10^6 cells/ml) and observed low but detectable signal (Figure 3A) with clearly visible attenuation of the intensity along the beam. The overall intensity is about 80 units, which corresponds to ~1/400 of the ADC range. Therefore, the use of at least 16-bit ADC is necessary in this case.

After 2-fold dilution of the sample, the scattering intensity decreased accordingly, and the attenuation almost disappeared, and the same effect was observed in subsequent dilution. Signal attenuation despite low side-scattering intensity means that large amount of energy is scattered in other direction (e.g. near-forward). However, the attenuation is not significant even for highest concentration used, which explains why the optical density is not used for counting of Jurkat cells.

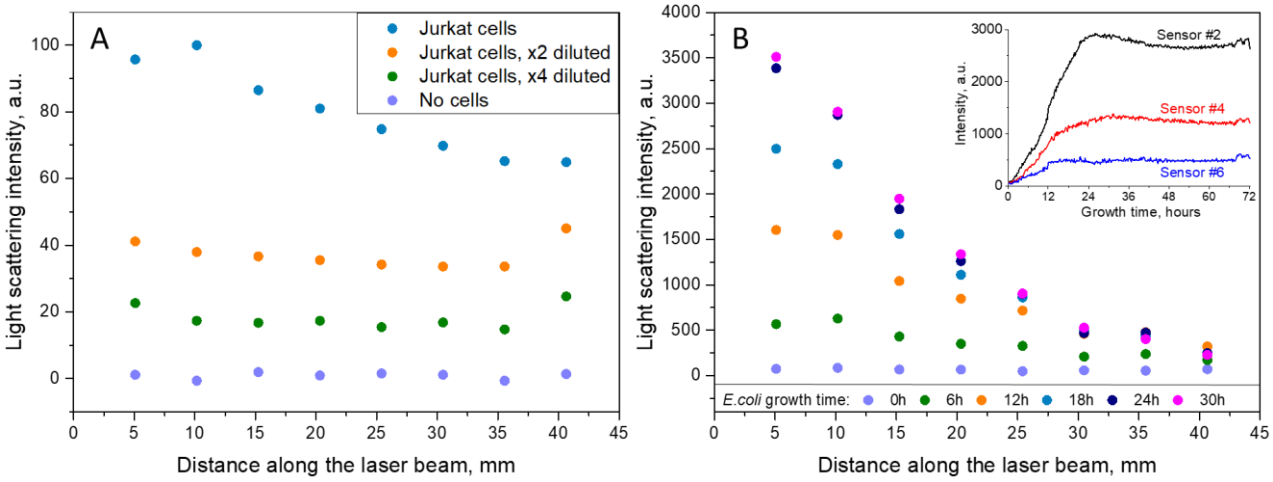

Figure 3. Testing of the prototype with cell cultures. A: Jurkat cells give low but detectable signal, but the attenuation of laser beam is visible only for maximal concentration (~10^6 ml^{-1}). B. Continuous monitoring of the *e.coli* bacteria growth.

In contrast, optical density is frequently used as a parameter reflecting the concentration of *e.coli* bacteria (14). These bacteria are rods with μm-ranged size and RI of ~1.4 (relative RI ~1.05) (15), and their concentration can reach 10^{12} cells/ml. Accordingly, the intensity of side-scattering and the attenuation of light is much greater than that for Jurkat cells.

One of the requirement for successful growth of bacteria culture is continuous stirring. Therefore, we implemented the simple shaker based on the servo motor which reversibly tilt the flask each 2 seconds. 1 ml of *e.coli* in a stationary concentration was diluted in 15 ml of LB medium and placed on the device for continuous monitoring of their growth. The device was placed in the laminar flow cabinet and programmed to perform light-scattering measurement each 15 min and transmit data to PC via Bluetooth. The results are shown in Figure 3B. As predicted, the intensity of scattered light is relatively high, as well as the beam attenuation. The inset shows the kinetics for three sensors, and different growth phases are clearly visible.

3. Conclusion

In this Letter, we present a device that is able to monitor the growth of a number of cells in a suspension non-invasively. The device uses a laser beam that pass through the culture flask and measures the intensity of scattered light as a function of coordinate along the beam. This optical scheme allows one to obtain accurate results for both high- and low-scattering samples. In experiments with Jurkat cell line and *e.coli* bacteria, using the portable prototype, we demonstrated the wireless monitoring of cell culture growth. Further development of this measurement principle and its application may facilitate the advancement of biomedicine and biotechnology.

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