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Detection of structural changes based on Mie-scattering analyses of mouse fibroblast L929 cells before and after necrosis

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ABSTRACT

The aim of the presented work is to investigate the angle-resolved scattering characteristics of biological nano- and micro-scaled cell structures. The scattering results of cellular structures were compared to measurements of ideal spherical nano- and micro-particles. A monolayer of mouse fibroblasts L929 cells was cultivated in a Dulbecco's Modified Eagle Medium (DMEM) in a standard 24 well cell culture plate. The system allows an in situ measurement directly in the standard cell culture plate and a contaminant-free investigation of the viability of the cell cultures. Of particular interest was whether changes in the tumor characteristics occur in necrosis or other cell-harming effects. Because of the size ratios between wavelength and the scattering particles, all observations were investigated using Mie scattering theory. A setup for reliable measurements was developed and the scattered angle dependent intensity obtained was compared with simulated scattering characteristics. A homemade supercontinuum (SC) light source was filtered by an optical bandpass filter with a central wavelength of 500 nm. The scattered portion of the pulsed SC light behind the sample was recorded in a time-resolved manner at defined angles. A specimen holder adapted to standard cell culture plates allows detection of scattered radiation at angles between $\pm 80^\circ$ without angle-dependent Fresnel reflection losses and a Snell's law bending of the propagation direction. Finally, the system was tested to detect structural changes of mouse fibroblast L929 cells before and after poisoning the cells with the cell detergent Triton X100 and the data clearly shows changes in the scattering characteristics when the cells were destroyed.

Keywords: scattering analyses, supercontinuum, L929 cells, Mie scattering

INTRODUCTION

The propagation of light in biological tissue is important for many diagnostic methods in the field of biomedical optics and biophotonics [1]. For these fields, optical performance is often limited by light scattering on cellular structures [2]. Biological tissue scatters light mainly in the forward direction with an angle-dependent intensity distribution dependent on the structure size, refractive index difference and geometry [3]. For the propagation of light in three-dimensional biological structures, complex transport equations can be determined [4]. A confirmation of structural changes in three-dimensional biological tissues is possible by different imaging techniques [1, 2, 5]. Measurements of elastic scattering of single cells allow a characterization of cellular structural changes [6, 7]. For many abnormal cell changes, an evaluation of structural changes in individual cells is not sufficient and a large number of cells must be investigated to verify results. Angular-dependent light scattering analyses in suspension of cells with a concentration between 1×10^5 to 2×10^5 cells/ml was realized to analyze cancer cell cycle phases [8]. For appropriate measurements, the cells were prepared accordingly and special cuvettes were used. A method of angle-resolved measurement of the scattered light to characterize pathological cell changes in standard cell culture plates would bring clear advantages.

EXPERIMENTAL ARRANGEMENT AND METHODS

Light scattering measurements: for angularly resolved light scattering measurements, a customized optical setup has been developed as seen in Fig. 1. For the setup, a fiber-coupled supercontinuum light source, developed and manufactured in cooperation with fiberware GmbH, was used [9]. The SC light source emits pulses with a full-width half maximum (FWHM) pulse width of 1.1 ns at $\lambda = 500$ nm and has a repetition rate of 8 kHz.

Figure 1. Setup used to measure angular-dependent light scattering. SC - supercontinuum light source, L1 - collimation lens, BS - non-polarizing beam splitter, D1 - reference detector, F - bandpass filter, P - polarizer, CCP - cell culture plate, SH - specimen holder, P - pinhole, L2 - collecting lens and D2 - avalanche detector and DU - detector unit.

The light of the SC - light source was spectrally filtered. A polarizer ensured perpendicular polarization. To capture the time-resolved signal independent of the signal strength, an optical trigger was used which enables the averaging of individual signals. A standard cell culture plate was placed on the sample holder and all of the 24 wells were examined. Through the use of immersion gel, the adapted sample holder can allow a measurement of the scattered components of the incident light in the angular range of $\pm 80^\circ$ without angle-dependent Fresnel reflection losses. By varying the position of detector unit, it is possible to capture the angular-dependent scattered light. The movement of the detector

unit was performed automatically by a motorized rotation stage. To capture the pulses of the SC-light source, an avalanche detector with a band width of 50 MHz was used. The detector shows a responsivity of 20 A/W at 500 nm. To determine the signal transfer function, the optical signal was attenuated with a neutral density filter by four orders of magnitude. The angle-resolved scattered light measurements were carried out in the investigated sensitivity range of the detector and a linear transfer function can be applied (see Fig. 2).

Figure 2. Signal transfer function the optical signal was attenuated with a neutral density filter by four orders of magnitude.

Cell culture: The L929 mice fibroblast cells were cultivated in a Dulbecco's Modified Eagle Medium (DMEM) / Nutrient Mixture Ham's F-12, which contained 10 % Fetal Calf Serum FCS and 1 % L-glutamine, in a standard 24 well cell culture plate. The cells were seeded in culture wells 24 hours before the measurement. The culture medium was incubated under the temperature of 37°C. The humidity was saturated and the CO₂ concentration was 5% in the incubator. The cell culture plates were removed from the incubator immediately prior to measurement and the measurement time was limited to thirty minutes.

RESULTS AND DISCUSSION

Of importance for the measurement method is the amplitude of the pulse since it is time-resolved over the angular measurement. In order to detect the amplitude of the pulse, the signals were numerically fitted with a Gaussian (see Fig.3a). Due to the bandwidth of the avalanche detector used, a temporal broadening of the pulse occurs. The pulse-to-pulse stability of the SC light source was recorded at a wavelength of 500 nm and shows a statistical variation and also was fitted with a Gauss-shaped distribution, as shown in Fig. 3b. The setup shows a standard deviation of $\pm 6.96\%$ as seen in Fig. 3b.

Figure 3. a) Measured signal of the SC light source with the avalanche detector and Gaussian fit. b) The pulse-to-pulse stability of the SC light source at a wavelength of 500 nm.

To qualify the measuring setup with angular resolution (Fig. 4), we used polystyrene beads in a water solution as a standardized sample to examine the scattering properties of the system. For these boundary conditions, Mie theory can be applied. The size uniformity of the polystyrene beads with a diameter of $1.6 \mu\text{m}$ was assumed to be $\pm 3\%$. The refractive index for the standardized polystyrene beads was set at $n = 1.603$ and for water $n = 1.335$ (both at 500 nm). The polystyrene bead solution was loaded into a standard 24-well cell culture plate to measure the angle-resolved scattering characteristics.

Figure 4. Measured angularly resolved signal of the polystyrene beads in a water solution and calculated according to Mie theory. The inset shows a microscope image of the polystyrene beads with a diameter of $1.6 \mu\text{m}$.

To show the possibility to analyze the change of cell components after necrosis based on scattering analyses, L929 mice fibroblast cells are killed by adding Triton X100. Through the addition of Triton X100 [10], the cell membrane is damaged and necrosis occurs. The cells are examined by light microscopes before and after necrosis, as shown in Fig. 5.

Figure 5. Mouse fibroblast L929 cells before a) and after necrosis b) by adding Triton X100.

The angle-resolved scattered light measurements of the medium, the living and the necrotic cells were repeated ten times at different wells of the cell culture plate. The averaged measured signals show a clear systematic change (Fig. 6). In the angular range between 17° to 63° , a reduction of the scattered light of up to 50% between living cells and cells after necrosis occurs. In the culture medium, there is considerably less light scattering.

Figure 6. Angular resolved measurement of the scattered light of Mouse fibroblast L929 cells before and after necrosis and the measured background of the cell culture medium. The mark I shows the reduction of the scattered light of up to 50% and II a shift in the scattered peaks after necrosis.

By destroying the cell envelope, the mitochondria are removed and that portion of scattered light disappears. The addition of Triton X100 significantly reduces the size of the cell nucleus, which leads to a shift in the scattering peaks to larger angles.

CONCLUSION

The presented method allows the study of structural cell changes of a large number of cells. The angular resolved measurement of the scattered light of polystyrene beads shows a very good agreement with the simulated data by Mie theory. The measurements were carried out in standard 24 well cell culture plates. The measurements were also made without affecting the cultivation of the cells under sterile conditions. Automated data logging enabled measurement times of less than five minutes. After necrosis, a reduction of the scattered light of up to 50% occurs in the angular range between 17° to 63°. The method could allow obtaining information on cell malignancy or morphological changes upon necrosis or apoptosis while averaging over several thousand cells during a measurement. This method provides a label-free noninvasive diagnostic.

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