VISUALIZING CELLULAR MICROSTRUCTURE BY LIGHT SCATTERING MICROSCOPY

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SUMMARY

Light scattering microscopy is suggested to provide some insight into cell architecture and to give information on cell malignancy or morphological changes. In particular, the angular resolution of Mie scattering provides valuable information about the microstructure of cells and their organelles (e.g. nuclear diameter), tissue architecture as well as changes related to necrosis or apoptosis. Since commonly used methods for measurements of elastic light scattering do not permit simultaneous visualization of sub-cellular structures, a conventional microscope was modified for scattering experiments with high spatial and angular resolution. Preliminary results indicate that elastic light scattering can be related to changes in cell morphology upon apoptosis and may further be helpful to distinguish various (e.g. malignant and non-malignant) cell types.

MATERIAL AND METHODS

In Fig. 1 a microscope setup is presented permitting scattering measurements of single cells, cell clusters and 3-dimensional cell spheroids with a resolution of ± 1°. Angular resolution is achieved by focusing a 470 nm laser diode into the aperture of the microscope objective lens (e.g. 40x / 1.30 oil immersion) after deflection by an adjustable mirror. Thus, the samples are illuminated by a parallel beam under variable angles. Scattered light is collected by the same objective lens and further focused by a Bertrand lens generating an image of the aperture plane. Within this image a central region of ± 1° is selected by a tiny aperture, so that backscattering can be measured in an angular range between slightly above 180° an d 230°.

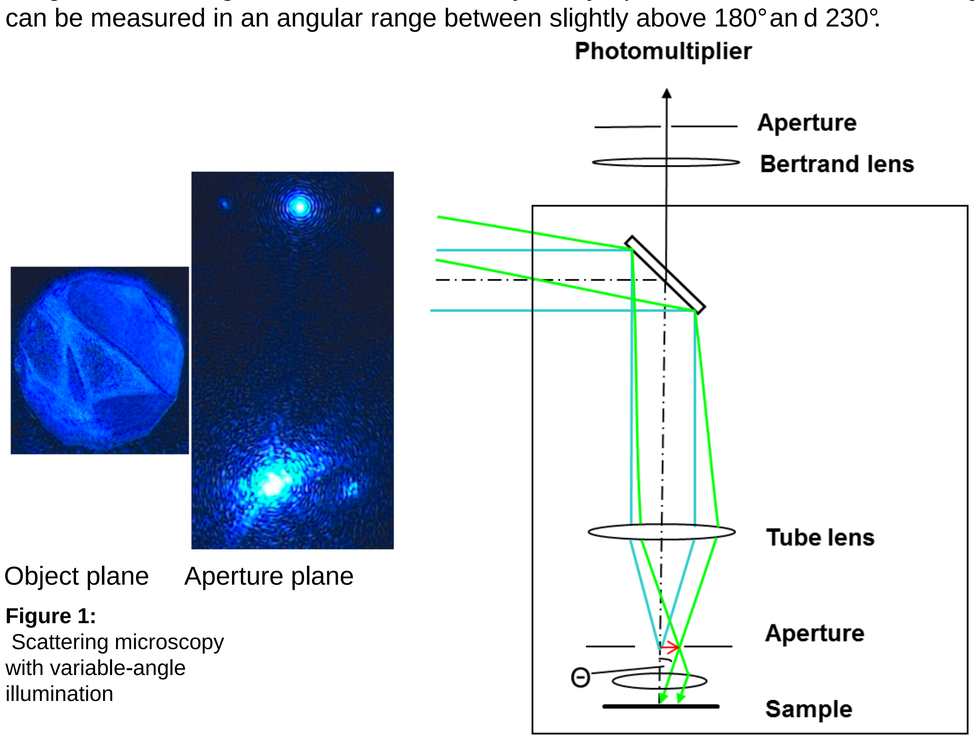

Figure 1:
Scattering microscopy with variable-angle illumination

SIMULATIONS

Figure 2 shows the simulated phase function for Mie scattering (non-polarized light) by single polystyrene beads with diameters of 2µm, 5µm and 10µm, assuming refractive indices of n_b= 1.59 for the beads and n_m = 1.334 for the surrounding medium (EBSS). Angular scattering representing these phase functions is periodic with a frequency increasing with the diameter of the scatterers. This can be proven by a Fast Fourier Transform (FFT) function.

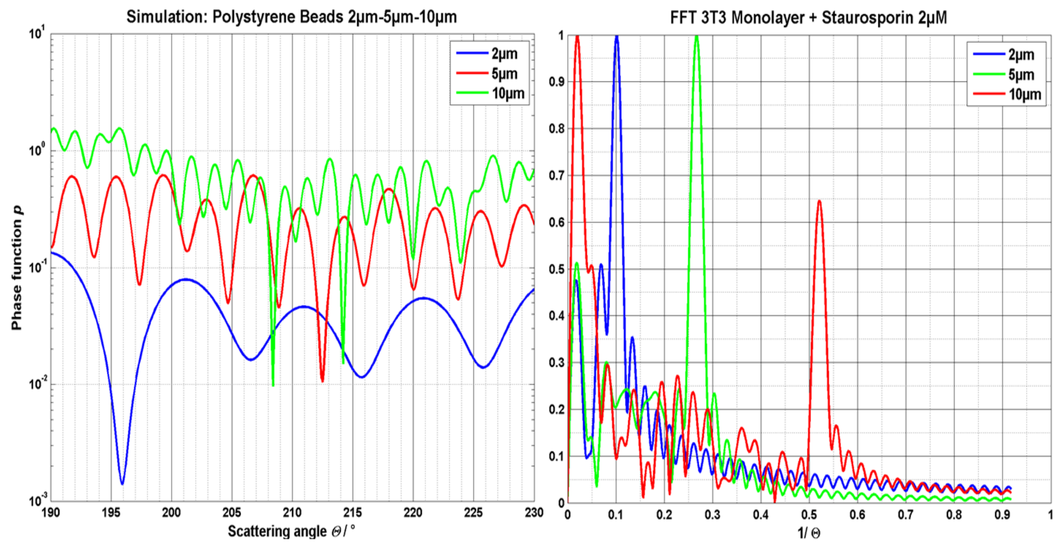

Figure 2:
Simulation of Mie scattering by polystyrene beads (left) and corresponding Fourier Transform (FFT) functions

RESULTS

Preliminary results indicate that elastic light scattering can be related to cell morphology in monolayers or spheroids. An example is given in Fig. 3 for 3T3 human fibroblasts prior and after apoptosis. In the latter case, increased intensity and oscillations of Mie scattering (Figure 3) reflect cell shrinking and formation of small vesicles. Scattering parameters may, therefore, give complementary information on cell metabolism and cell death.
In addition, light scattering may be useful to distinguish between tumor cells and non-malignant cells (Figure 4).

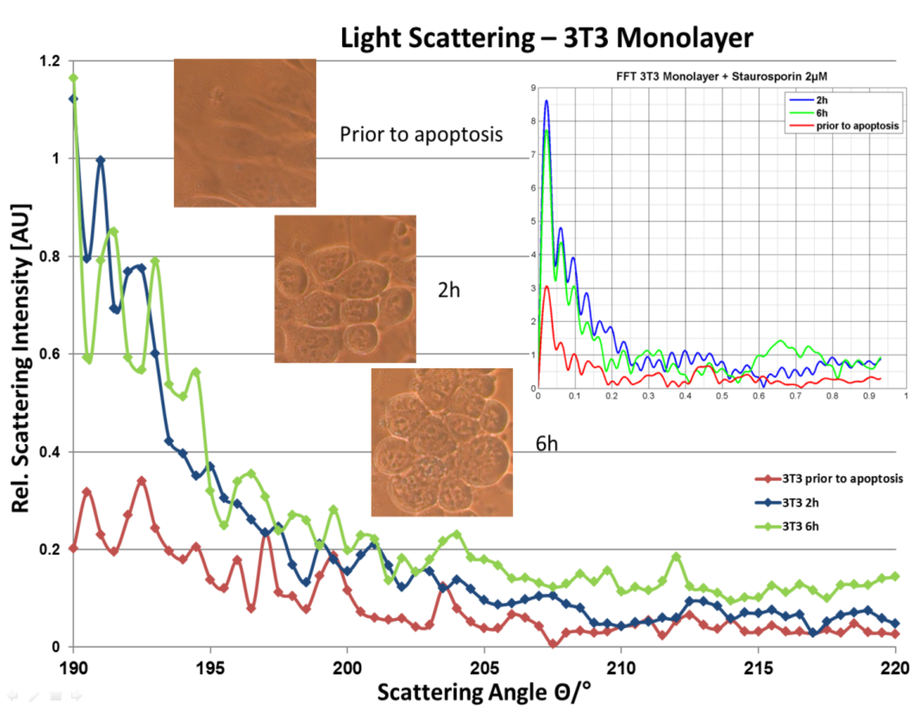

Figure 3:
Angular dependence of light scattering of 3T3 cells in the range of 190°-220°; red line: prior to apoptosis; blue line/green line: 2h/6h after incubation with 2µM Staurosporin (excitation wavelength: 470±5 nm; Plan-Neofluar 40x/1.3 microscope objective lens); Inlay: transmission microscopy and FFT.

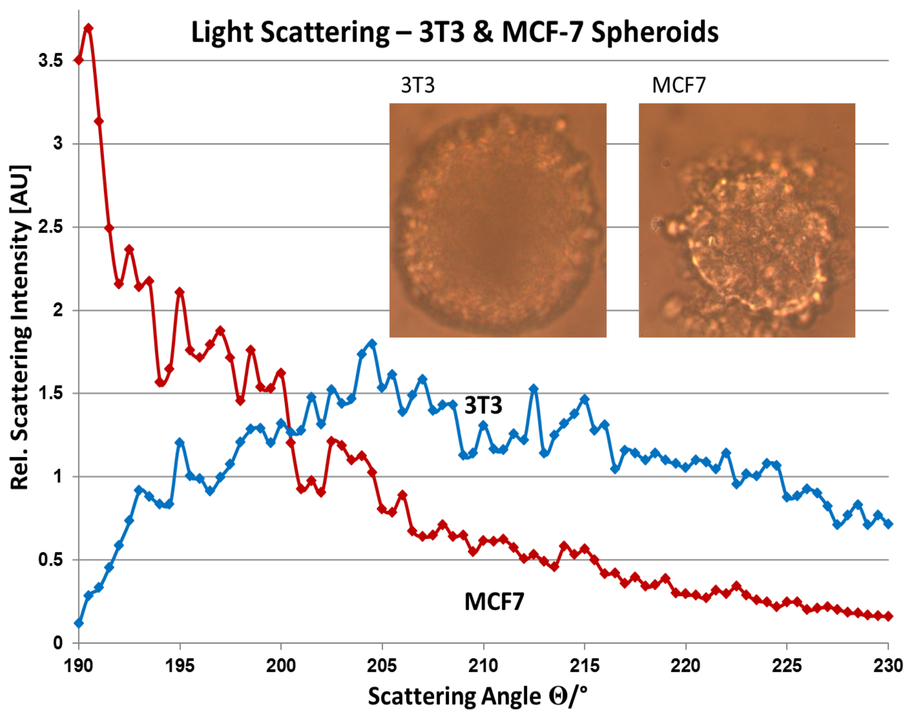

Figure 4:
Angular dependence of light scattering by MCF-7 breast cancer cells and 3T3 human fibroblasts (excitation wavelength: 470±5 nm; Plan-Neofluar 40x/1.3 microscope objective lens); Inlay: transmission microscopy.

ACKNOWLEGEMENT

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