The constituent parts of a cell, generally known as organelles, are at the resolution limit of optical microscopes. Dynamic alterations in organelle morphology can be important indicators of underlying biochemical activity in living cells. While these changes can be quantified by electron microscopy in specially prepared fixed (and therefore dead) cells, a microscopic technique better suited for live cell monitoring provides more insight into the biological process. Quantitative light scattering methods are an ideal way to complement existing microscopic techniques. These methods are noninvasive and sensitive to changes in the dimension and optical properties of particles with size on the order of the wavelength. In particular, angular scatter data has been used in cell and macromolecule analysis to probe intracellular morphology and obtain size information about objects that cannot be optically resolved.1-2 Such studies are spectroscopic and do not allow sample imaging. Typically, scatter source location information within the specimen is lost.

We recently demonstrated a technique that combines light scattering spectroscopy with imaging microscopy to track the location of morphologic change (and thus scatter change) within a monolayer of cells or even within a cell itself.3 This optical scatter imaging (OSI) technique produces images that directly encode a morphometric parameter within the microscope’s full field of view. The OSI method uses Fourier filtering to detect alterations in particle size with wavelength-scale dimensions. A “scatter ratio” image is generated by taking the ratio of images collected at high and low numerical aperture in central dark-field microscopy. Such an image spatially encodes the ratio of wide to narrow angle scatter and provides a measure of local particle size. We validated OSI on sphere suspensions and live cells. Unlike high resolution imaging methods, OSI provided size information for particles smaller than the camera’s spatial resolution.

The study of live cells undergoing programmed cell death—or apoptosis—achieved the most striking results. In this study, the OSI method quickly revealed apoptosis-induced subcellular changes that were not apparent in conventional differential interference contrast images (see Fig. 1). In light of the ongoing research and debate regarding the initial subcellular events involved in programmed cell death,4 OSI provides a novel perspective that cell biologists can use to track the dynamics of apoptosis—a process fundamental to biological homeostasis and development.

More generally, OSI is a simple and effective method to detect subtle in situ morphological changes for particles with wavelength-scale dimensions such as organelles and perhaps intracellular aggregates. The probed particles do not need to be individually resolved and measured by traditional morphometric methods. OSI thereby avoids the tedious process of image recognition, particle sizing, and counting. In contrast to electron microscopy and flow cytometry techniques, OSI requires no potentially damaging cell sectioning or staining procedures. OSI complements current microscopic methods and allows real-time monitoring of the same cells. The technique can be easily extended to high-throughput screening.

References

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