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Abstract. A dynamic light scattering technique is implemented using optical coherence tomography (OCT) to measure the change in intracellular motion as cells undergo apoptosis. Acute myeloid leukemia cells were treated with cisplatin and imaged at a frame rate of 166 Hz using a 1300 nm swept-source OCT system at various times over a period of 48 h. Time correlation analysis of the speckle intensities indicated a significant increase in intracellular motion 24 h after treatment. This rise in intracellular motion correlated with histological findings of irregularly shaped and fragmented cells indicative of cell membrane blebbing and fragmentation. [DOI: 10.1117/1.3600770]

Keywords: dynamic light scattering; optical coherence tomography; apoptosis; intensity autocorrelation; decorrelation time.

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In optical coherence tomography (OCT) images, speckle intensities depend on the number, size, optical properties and spatial distribution of scatterers within a resolution volume (RV). Imaging of living cells and tissues produces changes in the speckle pattern due to the motion of subresolution scatterers.[1,2] In addition to the presence of red blood cells flowing within the vasculature, scatterer motion in tissue can be caused by intracellular motion (IM). Examples include the movement of organelles along microtubules, the process of mitosis, and the morphological changes associated with apoptosis.

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During apoptosis, a predictable sequence of biochemical and morphological changes leads to cell death. This mode of cell death is essential in human development and homeostasis. Many cancer therapies take advantage of apoptosis in proliferating cancer cells to reduce tumor burden and cure patients. Morphologically, apoptosis is characterized by a rounding and shrinking of the cell, fragmentation of the nucleus and other organelles (e.g., mitochondria), membrane blebbing, and, ultimately, disintegration of the cell into intact membrane-bound fragments called apoptotic bodies.[3]

We hypothesize that the rate of IM in apoptotic cells will be higher than in viable cells due to the remodeling of the cytoskeleton required for membrane blebbing and cell fragmentation, and predict that this increase in IM can be detected using principles of dynamic light scattering adapted to OCT. In this letter we describe a technique to measure the decorrelation rate of speckle in multiple consecutive OCT images obtained using a swept-source OCT system with a 1300 nm light source.

Apoptosis was induced in acute myeloid leukemia (AML) cells (AML-5, Ontario Cancer Institute, Toronto, Canada) using the chemotherapeutic agent cisplatin, and cell pellets were imaged after 0, 2, 4, 6, 9, 12, 24, and 48 h of treatment. The treatment protocol and sample preparation techniques are described elsewhere.[4]

Optical coherence tomography data were acquired in the form of 14-bit interference fringe signals using a swept source OCT (OCM1300SS) system (Thorlabs Inc., Newton, NJ).[4] Two-dimensional frames containing 32 axial scans were recorded covering a transverse distance of 400 μm at a frame rate of 166 Hz. A region of interest (ROI) measuring 32 pixels in the transverse direction and 8 pixels in the axial direction was selected starting at 30 μm below the sample surface. For each pixel location, the signal intensity was plotted across all acquired frames. Region of interest selection and a representative signal intensity curve are shown in Fig. 1. Since the autocorrelation function and the power spectrum of a signal are Fourier transform pairs,[5] the autocorrelation of the time intensity signal at each pixel location was calculated by taking the inverse Fourier transform of its power spectrum. Treating the cell samples as nonergodic media, an intermediate scattering function (ISF) was calculated from the intensity autocorrelation function using the "heterodyne approach" described by Joosten et al.[6] This technique removes the contribution from the static scattering components to the fluctuating signal. An average decorrelation time (DT) was calculated for each data set by measuring the half width of the ISF at half its maximum value.

Histological sections (Fig. 2) obtained from fixed AML cell samples indicated significant structural changes after 24 h of cisplatin exposure. Nuclear condensation and fragmentation were observed, as well as irregular cell shapes, that may be indicative of cell membrane blebbing. Representative plots of the signal intensity as a function of time from a single pixel are shown in Fig. 2. The backscatter fluctuations from the samples treated for 24 and 48 h were higher in amplitude and frequency than at earlier times. This difference indicates more motion in samples exposed to cisplatin for 24 h and longer. The ISFs computed from the same four samples

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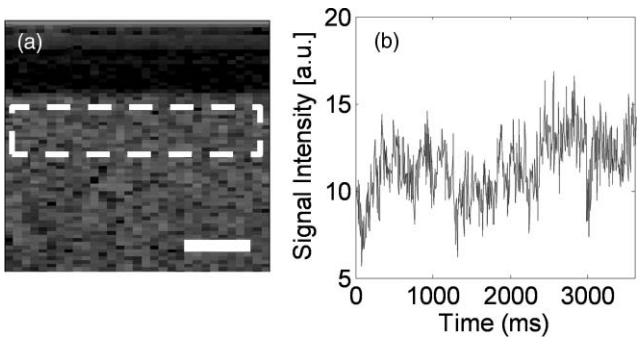

Fig. 1 Analysis methods. (a) OCT *b*-mode image of an AML cell pellet (scale bar = 100 μm) with analysis ROI outlined by a dotted line. (b) Signal intensity as a function of time for a single pixel in the ROI outlined in (a).

(Fig. 3) further confirmed this difference as the curves for samples treated for 24 and 48 h decayed more quickly. The DTs computed from cell samples obtained during two individual experiments are plotted as a function of treatment time in Fig. 3. Results from two separate experiments demonstrated good repeatability of this technique despite the biological variations inherent in such experiments. This graph indicated a significant drop in DT after 24 and 48 h of cisplatin exposure. The cell morphology observed in Fig. 2 suggests that these measurement times correspond to the stage in the apoptotic process where cell membrane blebbing and fragmentation occurs. We hypothesize that the significant drop in DT is related to an increase in IM caused by cytoskeletal and membrane structural changes and reorganization required for this fragmentation.

The resolution volume of our OCT system is approximately the size of a single cell. Scatterers giving rise to the signal intensity in each RV may include organelles, nuclear material, cytoskeletal components, and the cell membrane. Any change in the spatial distribution and scattering strength of these components can introduce fluctuations in the speckle intensity. Events that could modify the scatterer spatial distribution and scattering strength include movement or reorganization of the scatterers within the RV or the arrival and departure of scatterers into and out of this volume. A cell's contents are continuously moving due to various forces. Motion may be driven by active processes such as organelle transport or cytoskeletal restructuring during mitosis and apoptosis.[7] Diffusive transport of small organelles, vesicles, and macromolecules is also present due to thermal processes (Brownian motion), as well as from the fluctuation of the cytoplasm caused by movement of motor-bound organelles and the cytoskeleton.[8]

Assuming the dominant optical scatterers inside living cells are the mitochondria[9] and the nucleus,[10] we expect to see a change in the rate of IM during apoptosis due to mitochondrial and nuclear fragmentation. Furthermore, nuclear and mitochondrial fragments inside a cell will be subject to cytoplasmic motion caused by contractile forces of the cytoskeleton during membrane blebbing and the formation of apoptotic bodies. The period between the induction of apoptosis and the first morphological signs of cell death is asynchronous across a given population of cells and ranges between 2 and 48 h. The duration of the execution phase (the period during which structural changes occur), however, is largely invariant and lasts approximately 2 to 4 h.[11] Thus, the entire process of cell shrinkage, nuclear fragmentation, membrane blebbing, and the formation of apoptotic bodies occurs over a relatively short time in a given apoptotic cell. It is, therefore, not surprising to see a significant drop in DT during apoptosis, indicating an increase in IM.

Changes in cell mechanical properties leading to variations in the rate of Brownian motion or a reduction in the signal-to-noise ratio (SNR) due to a decrease in scattering intensity could also cause a drop in the DT. A particle tracking microrheology study conducted by El Kaffas[12] indicated a stiffening of the intracellular environment during apoptosis. This would reduce

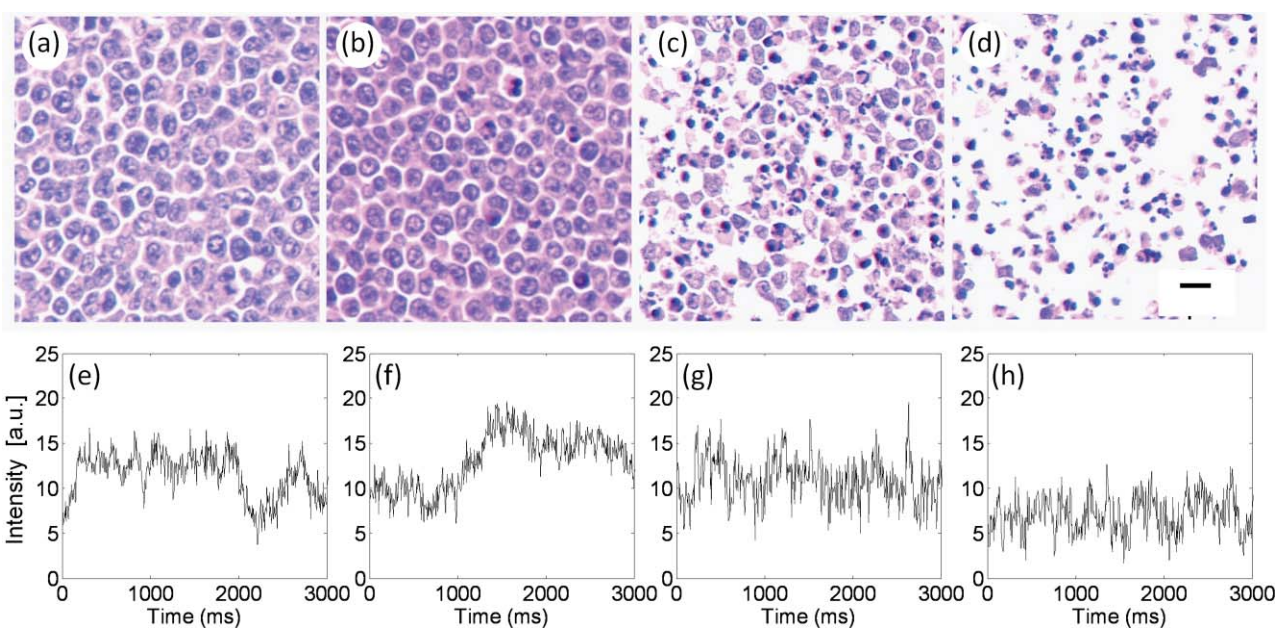

Fig. 2 Hematoxylin and eosin (H&E) stained sections obtained from cisplatin treated cells after (a) 0 h, (b) 12 h, (c) 24 h, and (d) 48 h of treatment. The scale bar represents 10 μm. Representative signal intensity curves from a single pixel at (e) 0 h, (f) 12 h, (g) 24 h, and (h) 48 h.

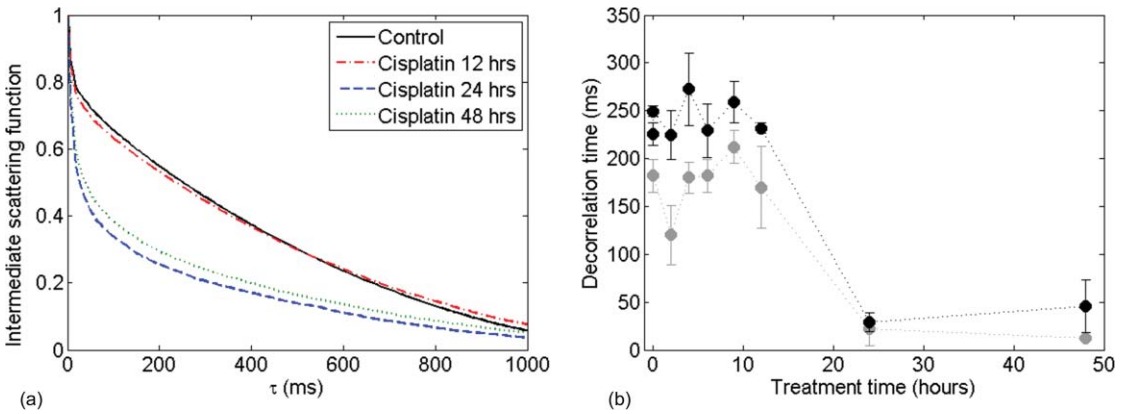

Fig. 3 (a) Average intermediate scattering functions computed from a selected ROI in AML cell pellets. (b) Decorrelation time computed from AML cell samples treated with cisplatin over a 48 h period. Each curve corresponds to a separate experiment. Error bars represent the standard deviation of 10 separate measurements from each sample.

Brownian motion within the cells and would lead to an increase in DT. Therefore, we do not believe changes in cellular mechanical properties to be responsible for the significant decrease in DT observed at 24 and 48 h of cisplatin treatment. Furthermore, we have previously shown that AML cells undergoing apoptosis induce an increase in OCT backscatter intensity,[4] making a reduction in SNR unlikely.

Several simple classical models exist for calculating the dynamic light scattering properties of systems of particles in motion.[13] These include models for the random (Brownian) motion of spherical particles suspended in a liquid medium, the uniform motion of particles subjected to an external force (flow), and the complicated movement of motile micro-organisms. The motion inside living cells is far more complex than any of the existing models, not only because of the various sources of IM, but also due to the large variation in size of subcellular components. A theoretical treatment of the dynamic light scattering properties of cells would likely include a combination of the above-mentioned models. We hypothesize that the shape of the ISF depends on the motion of the dominant scatterers in our sample. Further investigation of the relationship of cell viability to the shape of the ISF is the subject of future work.

We have demonstrated through an apoptosis time course experiment that our technique is repeatable and sensitive to variations in IM related to cell death. Since this dynamic light scattering technique relies on signal fluctuations rather than the absolute value of the signal intensity, effects of signal attenuation and scattering angle are greatly reduced. For this reason we believe this method provides an advantage over techniques measuring backscatter strength for cell death detection.[4] On the other hand, this method is more sensitive to the effects of bulk motion and blood flow *in vivo*. We are currently investigating methods to segment vascular structures and correct for motion artifacts to allow for *in vivo* implementation of this technique.

In summary, we have adapted concepts from dynamic light scattering and applied them to OCT to obtain measures of IM and have demonstrated that this method can reliably detect changes in the rate of IM between viable and apoptotic cells *in vitro*. To our knowledge, this is the first time that dynamic light scattering has been applied to OCT for detecting apoptosis. Currently, feasibility studies are underway to investigate the implementation of this technique *in vivo* for detecting cell death in a mouse tumor model.

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