

Speckle Decorrelation as a Method for Assessing Cell Death

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Abstract: A speckle decorrelation rate was measured in OCT images of cell spheroids at various stages of growth. The decorrelation rate was related to the extent of cell death observed in histological sections of spheroids.

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1. Introduction

Apoptosis is a process by which a predictable sequence of biochemical and morphological changes lead to cell death. This form of cell death is essential in human development and homeostasis. Many cancer therapies take advantage of this phenomenon to induce cell death in proliferating cancer cells in an attempt to kill tumor cells and cure patients. Morphologically, apoptosis is characterized by rounding and shrinkage of the cell, fragmentation of the nucleus and other organelles, membrane blebbing and ultimately disintegration of the cell into small fragments called apoptotic bodies [1].

Speckle in optical coherence tomography (OCT) images results from light backscattered by multiple scatterers within a resolution volume [2]. The intensity of speckle is dependent on the number, size, optical properties and spatial distribution of scatterers. Imaging of living cells and tissues produces changes in the speckle pattern due to the motion of subresolution scatterers [3, 4]. Scatterer motion in this case can be caused by the movement of organelles along microtubules necessary for processes involved in cell homeostasis, the reorganization of a cell's contents in preparation for mitosis, the process of mitosis itself and the morphological changes which occur during apoptosis. We hypothesize that the rate at which the speckle pattern will change varies between viable cells, cells at various stages of apoptosis, and nonviable cellular debris.

We demonstrate, here, a technique to measure the rate of decorrelation of speckle in multiple consecutive OCT images. We have applied this technique to the imaging of human colorectal adenocarcinoma multicellular tumor spheroids (MCTS). The tumor spheroid is an *in vitro* three dimensional cell culture system often used to model the *in vivo* growth properties of tumors. These cell aggregates are typically composed of a core of quiescent cells surrounded by an outer rim of proliferating cells. As the spheroid grows beyond 400-500 μ m in diameter a central zone of cell death begins to form as a consequence of nutrient deprivation caused by the inability of oxygen and growth medium to penetrate to its core. This region progresses to necrosis with continued spheroid growth [5]. It has been demonstrated that cell death in the core of HT-29 MCTS is induced by apoptosis [6].

2. Materials and methods

All cell work was conducted using HT-29 cells (ATCC: HTB-38), a colorectal adenocarcinoma cell line. Cells were maintained in McCoy's 5A modified medium (Sigma-Aldrich, St. Louis, MO) and supplemented with 5% fetal bovine serum and 1% streptomycin. Multicellular tumor spheroids were grown by inoculating approximately 10^6 cells in 15 mL of medium into a sterile 100 mm petri dish. After a period of 5-6 days cell aggregates were transferred to a new petri dish and medium was refreshed every 2-3 days. As they grew in size, the number of spheroids per dish was reduced to maintain a constant cell to growth medium ratio. Spheroids were imaged after 3 weeks and 6 weeks of growth. Immediately after imaging, each spheroid was fixed in 10% formalin for 24 hours and subsequently paraffin embedded and sectioned for haematoxylin and eosin (H&E) staining.

Optical coherence tomography images and data were acquired using a Thorlabs Inc. (Newton, NJ) swept source OCT (OCM1300SS) system. The light source on this system has a 100 nm bandwidth centered at 1325 nm with a coherence length of 6 μ m and a mean beam spot size of 13 μ m in the focal field. Data were collected in the form of 14-bit OCT interference fringe signals. For each spheroid, OCT data were collected over a period of 1 minute in a single imaging plane. At every pixel location in the OCT image, the intensity was plotted as a function of time using frames spaced 1 second apart. An autocorrelation function (AF) was calculated for the resulting curve and the distance (in time) between the peak of the AF and the point where it decayed to half of the peak value (the half

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width at half maximum, HWHM) was assigned to the pixel. This parameter indicates how quickly the signal intensity at each pixel decorrelates. The HWHM values were used to generate a decorrelation map (DM) by assigning the values to the “hot” colormap in the numerical computing software MATLAB (Mathworks, Natick, MA), with smaller values corresponding to the black end of the color bar and higher values to the white end. For pixels where the intensity is changing rapidly as a function of time, the width of the autocorrelation will be small, as the autocorrelation curve decays more rapidly. This will result in a darker color on the DM.

3. Results

Optical coherence tomography images of 3 week and 6 week old spheroids are shown in Fig. 1 (top row). The 6 week spheroid is significantly larger and, due its size, acquires a flattened shape when placed on a layer of agarose gel for imaging. Note that prior to imaging, when suspended in growth medium, both spheroids shown in Fig. 1. maintained a spherical shape.

B-Mode OCT images show a region of increased scattering at the core of both spheroids. We have demonstrated previously that an increase in backscatter can be correlated with cell death (manuscript in progress). The DM of the 3 week old spheroid revealed a region at its centre with values 49% lower compared to its edges. This suggests that intracellular motion was higher in the core of the spheroid as compared to regions near the edge. The DM of the 6 week old spheroid reveals a different trend. The core of this spheroid had values 71% higher as compared to the edge region which was narrower compared to the spheroid imaged at 3 weeks. This suggests very low intracellular motion at the core of the older spheroid and this region of low motility extends much closer, if not completely, to its edges.

Haematoxylin and eosin stained sections (Fig. 2) indicated a very different state of cell viability between the two spheroids. The 3-week old spheroid has a rim of viable cells with patches of necrosis beginning to form at its core. The necrotic regions were characterized by pyknotic and/or fragmented nuclei, cell shrinkage and blebbing of cellular membranes. The older spheroid had an entirely necrotic core in which very few if any viable cells can be found. The edge of that spheroid contained cells that seemed to be adherent, however the nuclei were pyknotic and had lost their round shape indicating the possibility that these cells were likely beginning to die as well .

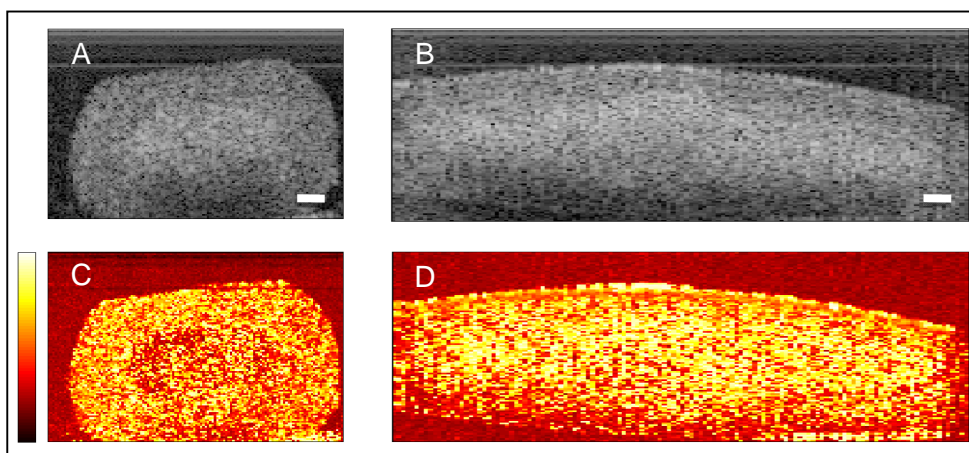


Fig. 1. Optical coherence tomography images (A, B) and decorrelation maps (C, D) of HT-29 multicellular tumor spheroids. The spheroids depicted in the left column (A, C) and the right column (B, C) have undergone 3 weeks and 6 weeks of growth, respectively. Color bar corresponds to the half width at half maximum value of the autocorrelation of pixel values as a function of time. Scale bars represent 100 μ m.

4. Conclusions

We have demonstrated a technique based on the measure of cellular motion for assessing the extent of cell death. Changes in decorrelation of speckle intensity as a function of time were observed between spheroids at different stages of growth. These changes were correlated with structural differences observed in histological sections which were related to the size of the necrotic regions and the stage of cell death in the core of the spheroids. This study shows the potential of using such a technique in conjunction with the measurement of backscatter intensity changes to assess the progression of cell death in tumor models.

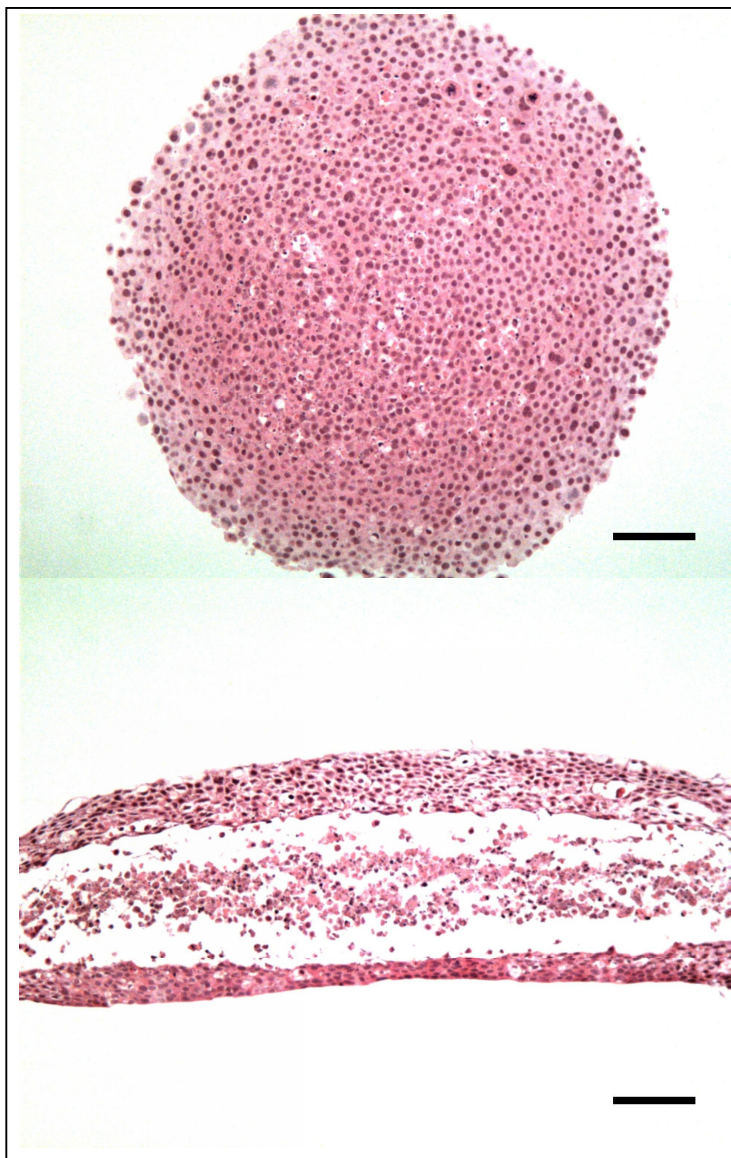


Fig. 2. Haematoxylin and eosin stained sections of 3 week old (top) and 6 week old (bottom) HT-29 multicellular tumor spheroids. White spaces are a histological retraction artefact due to cell death. Scale bars represent 100 μ m.

5. References

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