Measuring Intracellular Motion in Cancer Cells using Optical Coherence Tomography

Azhar Zam*[1,2,3] and Michael C. Kolios[1,2,3]
[1]*Department of Physics, Ryerson University, Toronto, ON M5B 2K3, Canada*
[2]*Institute for Biomedical Engineering, Science and Technology (iBEST), a partnership between Ryerson University and St. Michael's Hospital, Toronto, ON M5B 1W8, Canada*
[3]*Keenan Research Centre for Biomedical Science of St. Michael's Hospital, Toronto, ON M5B 1W8, Canada*
*azharzam@ryerson,ca

ABSTRACT

In this study, we demonstrate that OCT speckle decorrelation techniques can be used to probe intracellular motion in cancer cells. Spheroids and cell pellets were used as a model to probe intracellular motion. $ZnCl_2$ was used to inhibit mitochondrial motion within the cells. The results reveal the changes in intracellular motion during the spheroid growth phase. Moreover, to modify the motion of mitochondria, cell pellet were exposed to $ZnCl_2$, and agent known to o impair cellular energy production through inhibition of mitochondrial function. The speckle decorrelation time during the growth phase of spheroids decreased by 35 ms over 21 days and 25 ms during inhibition of mitochondrial motion 10 minutes after exposure to $ZnCl_2$.

Keywords: Optical coherence tomography; Intracellular motion; Cancer cells; Mitochondria; Speckle decorrelation; Motility map;

1. INTRODUCTION

Intracellular motion represents the dynamics of subcellular structures which are important marker of cell health and metabolism. Measuring and imaging intracellular motion in cancer cells is a very efficient way to test their response to drugs. The typical way drugs are tested, in 2D cell cultures, often has different cellular properties to tissue, thus more sophisticated models like cell spheroids have been developed.

Optical coherence tomography (OCT) is an emerging biomedical imaging technique that can perform cellular-resolution imaging in situ and in real-time. Dynamic light scattering OCT (DLS-OCT) [1] and Full field OCT (FF-OCT) [2] have been used in the past to explore and measure the dynamic properties of subcellular structures. Previous work from our lab has used DLS – OCT to observe long term phenomena of cell apoptosis in cancer cells [3].

Targeting mitochondrial metabolism to treat cancer has been investigated [4]. The availability of an imaging system to monitor treatment efficacy will be a great advantage. In this paper, we present a method using OCT speckle decorrelation and the use of motility maps to monitor intracellular motion in cancer cell growth phase (using spheroid models) and to monitor the inhibition of mitochondrial motion inside cancer cells.

2. MATERIAL AND METHODS

The OCT System

The longitudinal acquisition of in situ cell spheroid and cell pellet B-Scans and volumetric scans were done using a high speed MEMS-VCSEL Swept Source OCT from Thorlabs (see Figure 1). The Swept Source Laser

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has 100 nm FHWM at 1300 nm central wavelength and operating at 100,000 A-scan/s. The OCT system axial resolution is ~12µm and lateral resolution is ~ 25µm. The OCT images (B-scans) consist of 100 A-scans (lines) acquired over 0.5 and 1 mm to provide a high frame rate (500 Frames/s acquisition speed) required for our analysis. The volumetric scans were acquired in repetitive 3D and Speckle Variance over a time period of 21 days. The volumetric scans were also acquired in repetitive 3D and Speckle Variance for before and after exposure to $ZnCl_2$.

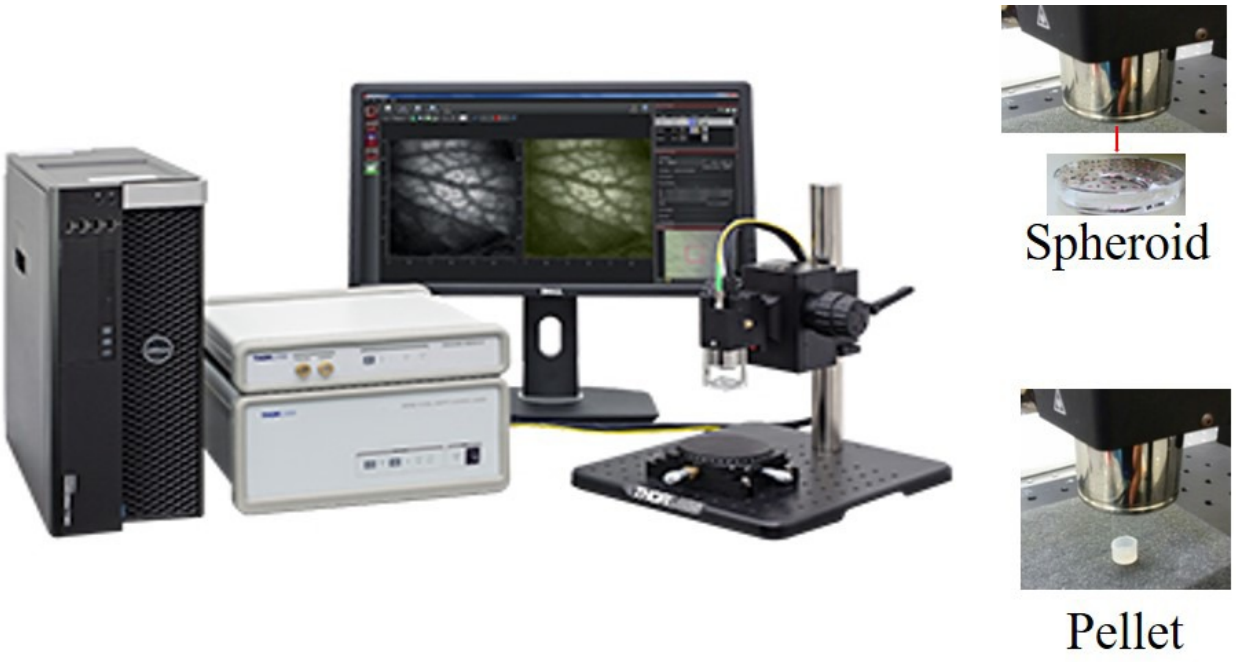


Fig. 1. High speed MEMS-VCSEL Swept Source OCT from Thorlabs. Scanning protocols were adjusted accordingly to enable the intracellular motion analysis for both spheroids and pellets.

OCT Speckle decorrelation and Motility map

The OCT speckle decorrelation method was used to calculate the decorrelation time [3]. Two-dimensional frames containing 100 axial scans were recorded covering a transverse distance of 500 µm at a frame rate of 500Hz. A region of interest (ROI) measuring 98 pixels in the transverse direction and 10 pixels in the axial direction was selected starting at 30 µm below the sample surface. A time fluctuation signal from the ROI was obtained by subtracting the signal mean and dividing by the standard deviation. The autocorrelation of the time intensity signal was calculated by taking the inverse Fourier transform of its power spectrum.

The AC function from a suspension of monodisperse spherical particles undergoing Brownian motion decays exponentially and is described by the following equation for a homodyne system:

$$A(\tau) = e^{-2q^2 D_B t}$$

where τ is the decorrelation time, $q = \frac{4\pi}{\lambda}$ and D_B is the diffusion coefficient. An exponential was fit to an average AC function obtained from the selected ROI and the τ was extracted using this equation.

The OCT motility map method was used to monitor and visualize the intracellular motion within clusters of cancer cells with the resolution volume of the OCT instrument. The motility map was calculated based on the following equation:

$$Motmap_{ijk} = \frac{\frac{1}{N-1}\sum_{i=1}^{N}\sqrt{\left(I_{ijk} - I_{mean}\right)^2}}{I_{mean}} \qquad (2)$$

where N is the number of the Volumes collected and j and k are axial and transverse pixels in the image. The higher value of Motmap shows faster intracellular motion or higher intracellular dynamics.

3. RESULTS AND DISCUSSION

The OCT decorrelation time was calculated for longitudinal study of cancer cell growth phase. Fig 2 shows the autocorrelation function and decorrelation time over 21 days.

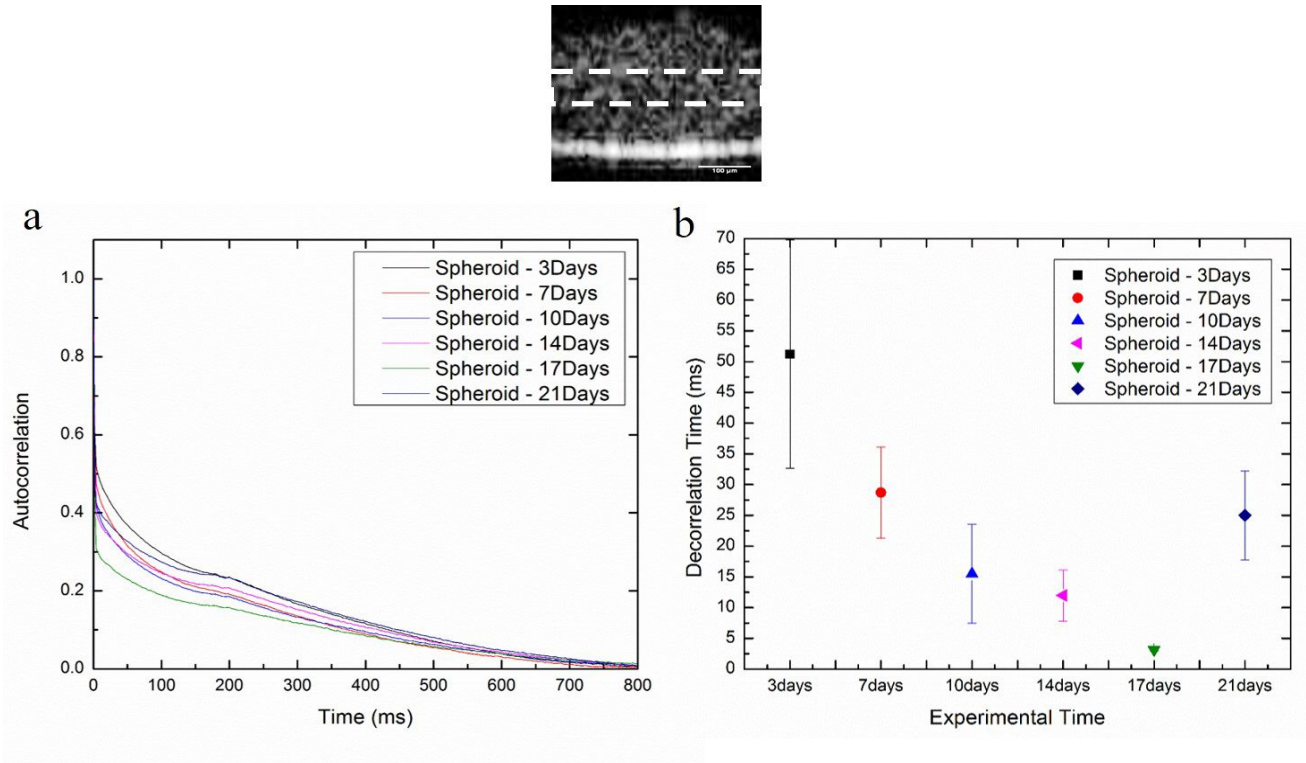


Fig. 2. a) OCT autocorrelation function and b) OCT decorrelation time of cancer cell over 21 days. White dash line on OCT image shows the region of interest (ROI).

The OCT motility map was also acquired for 17 days and 21 days to visualize the intracellular motion at these time points (see Fig 3).

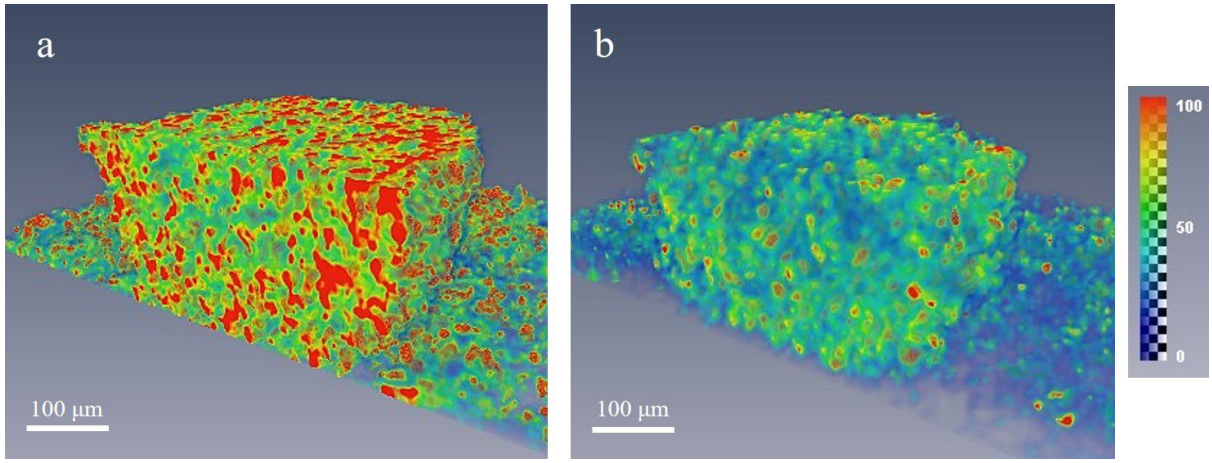
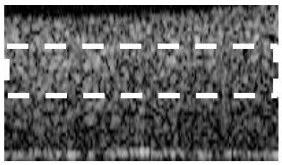


Fig. 3. OCT motility map acquire at a) 17 days and b) 21 days. Color bar shows the intracellular motion with red as highest motion and blue as lowest motion.

The OCT decorrelation time was calculated also in the experiments related to the inhibition of mitochondrial motion of cancer cells. We used $ZnCl_2$ to inhibit the mitochondrial motion of cancer cells. Fig 4 shows the autocorrelation function and decorrelation time for cancer cell before and after expose to $ZnCl_2$.



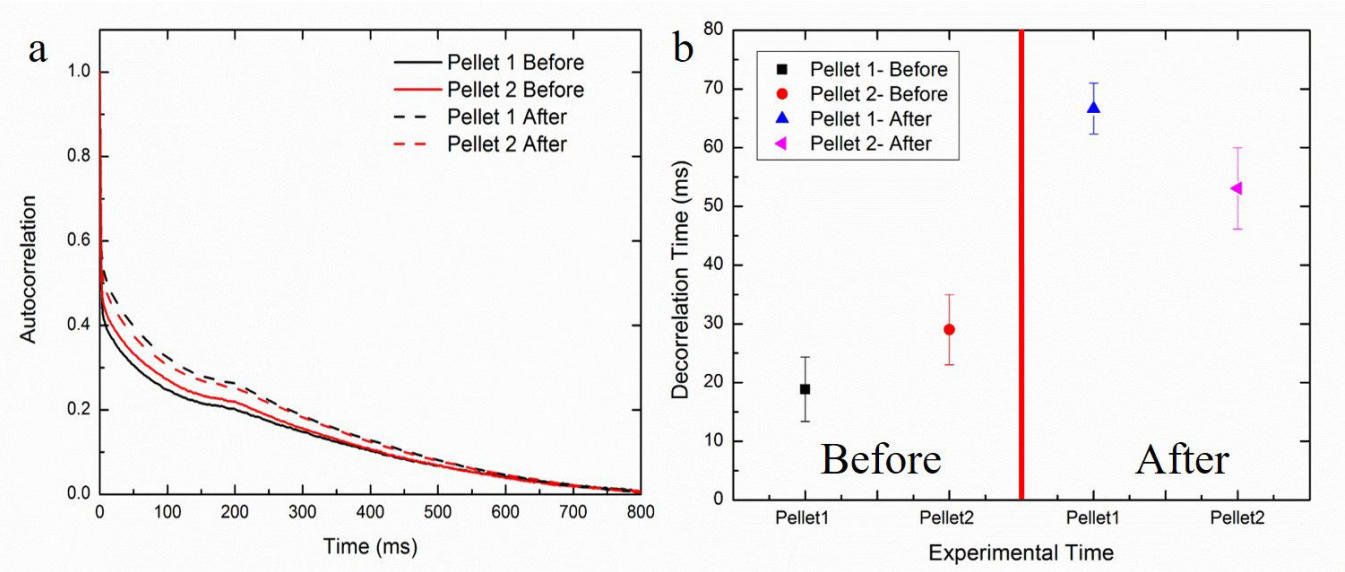


Fig. 4. a) OCT autocorrelation function and b) OCT decorrelation time of cancer cell before and after expose to $ZnCl_2$. White dash line on OCT image shows the region of interest (ROI).

The OCT motility map was also acquired for before and after expose to $ZnCl_2$ to visualize the intracellular motion before and after inhibition of the mitochondrial motion (see Fig 5). $ZnCl_2$ has been used in previous studies to inhibit mitochondrial motion [5].

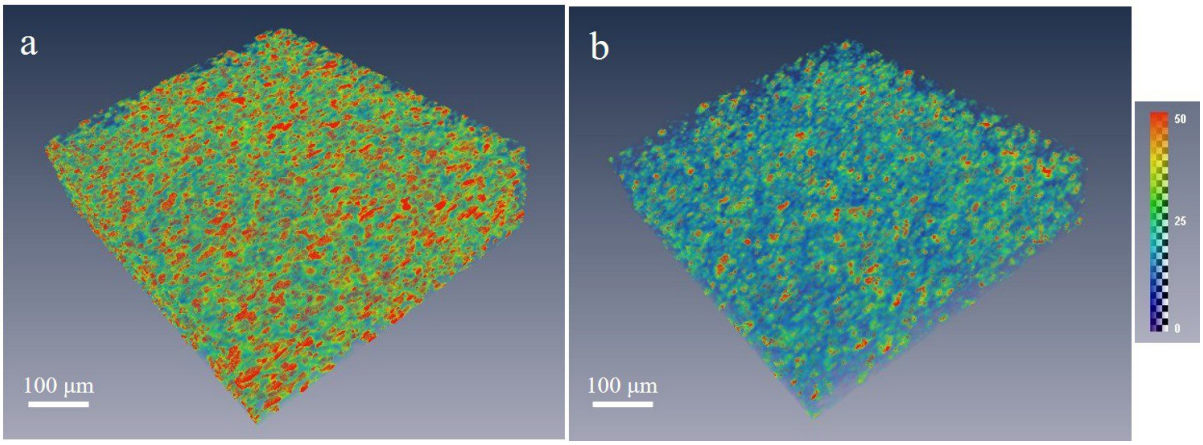


Fig. 5. OCT motility map acquired a) before and b) after exposure to $ZnCl_2$. Color bar shows the intracellular motion with red as highest motion and blue as lowest motion.

4. CONCLUSIONS

We have presented speckle decorrelation and motility map analysis of the cancer cell spheroid and pellets. The speckle decorrelation time during the growth phase of spheroids decreased by 35 ms over 21 days and 25 ms during inhibition of mitochondrial motion 10 minutes after exposure to $ZnCl_2$. The motility images show that the cancer cells after exposure to $ZnCl_2$ are less active and have lower metabolic activity due to inhibition of mitochondrial motion. We verified inhibition of mitochondrial motion after exposure to $ZnCl_2$ using confocal microscopy and we also found that inhibition of mitochondrial motion happened after exposure to $ZnCl_2$. Our finding shows early results and feasibility of using OCT speckle decorrelation and motility maps for monitoring the efficacy of mitochondrial targeted cancer therapy.

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