# OCT-Based Quantification of the Effect of a Drug on the Motility of Mammary Organoids

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**Abstract:** We quantified the effect of doxorubicin on the motility of mammary epithelial cells in 3D cultures by Optical Coherence Tomography. The measured cellular motility decreased in a time-dependent fashion after exposure to doxorubicin.

**OCIS codes:** (170.4500) Optical coherence tomography; (170.3880) Medical and biological imaging

## 1. Introduction

There is a need for technologies to predict the efficacy of breast cancer treatment, and the ability to measure the response of breast cancers to potential therapies may lead to new treatment options [1]. There is also a need for non-invasive, quantitative assays that can be applied to 3D models [2]. We propose a quantitative method for measuring the motility of mammary organoids in 3D cultures based upon Optical Coherence Tomography (OCT). We employ OCT to collect repetitive, non-invasive images of mammary organoids *in situ*, which is used to quantify cellular motility.

Here we investigate the metrics of inverse power law and fractional modulation amplitude [3] applied to the speckle fluctuation spectra of pre-malignant mammary epithelial cells (MEC) exposed to doxorubicin (Dox), a DNA damaging toxicant. We show that the power-law exponent and the motility amplitude are well-behaved in these cultures and are invariant to system SNR and image depth. This method provides new insight into the effect of the drug on epithelial cell motility.

## 2. Methods

A custom, spectral-domain OCT system has been utilized for imaging [4, 5]. Briefly, the system consists of a Ti:Sapphire laser centered at 800 nm and a custom spectrometer with a 25 kHz Dalsa Piranha line scan CCD camera, operated at 2 kHz for this study. The resolution is ~3 × 12 μm axial × transverse. A frame rate of 1 Hz is employed to emphasize cellular dynamics occurring on the time scale of several seconds [5], and typically 300 frames are collected per time series.

MECs which form organoids over time recapitulate many features of breast ducts *in vivo* such as luminal-basal polarization. Pre-malignant MECs were MCF10DCIS.com which were seeded into artificial extracellular matrix (ECM) comprised of 1:1 collagen I: Matrigel at 30 cells/μL and incubated for 7-10 days until organoids were formed, at which time they were treated with Dox at three different concentrations (0 μM, 1 μM and 10 μM). OCT imaging was performed of Dox-treated cultures at 0, 1 hr, 24 hrs, 48 hrs and 6 days after treatment.

Power spectral analysis of OCT fluctuations is illustrated in Fig. 1 (top). Briefly, the power spectral density in the frequency domain $S(f)$ at each pixel was first obtained from the Fourier transform of the measured power spectrum. Then, we averaged over pixels in the region of interest (ROI) and fitted $S(f)$ using the inverse power law model: $S(f) = I_0 f^{-\alpha} + n$, where $\alpha$ is the exponent, $I_0$ is the DC power of the OCT signal, and $n$ is white noise (shot noise). Examples of ROIs and model fittings are shown in Fig. 1 (bottom). The frequency decay exponent, $\alpha$, is sensitive to functional changes in the MECs. The increase in $\alpha$ is indicative of lower fluctuation intensity at higher frequencies. We also performed a control test on the stability of this metric and proved that it is independent of the system SNR and image depth.

A complementary metric of fractional modulation amplitude to quantify the strength of the fluctuations is also employed: $M = \frac{\sqrt{\Gamma(\Delta t) - \langle S_{OCT} \rangle^2}}{\langle S_{OCT} \rangle}$, where $M$ is the motility amplitude, expressed as a modified standard deviation that is normalized by pixel intensity (unitless). Instead of using $S_{OCT}^2(t)$, the autocorrelation function, $\Gamma$, is used, $\Gamma(\Delta t) = \frac{1}{N-1}\sum_{i=2}^{N} S_{OCT}(t_i) S_{OCT}(t_{i-1})$, which is the autocorrelation at $t=\Delta t$ (sampling time). The use of $\Gamma$ effectively omits

shot noise that decorrelates instantaneously. A control test was also performed and this metric was proven to be similarly stable as a function of the system SNR and image depth.

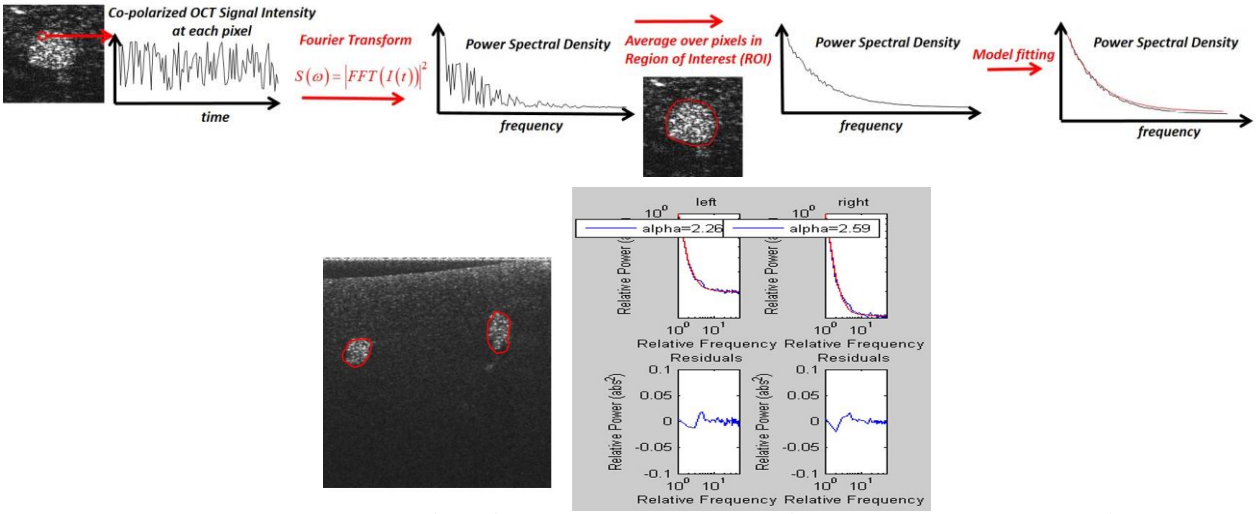


Figure1. (Top) Power spectral analysis of OCT fluctuations; (Bottom) Examples of ROIs and inverse power law model fittings.

## 3. Results

Figure 2 shows the time- and dose-dependent response of MEC organoids in 3D culture to Dox. The increase in $\alpha$ (left) along time at each dose of Dox indicates lower fluctuation intensity at higher frequencies, which is attributed to cell death during Dox treatment that eliminates cellular motility. The decrease in $M$ (right) after the exposure to Dox resulted in lower motility amplitude, and decreased in a time-dependent fashion. Statistically, $p$-values are less than 0.001 at and above 1 hour for $\alpha$, compared to the control data (time $t$=0), but require about 24 hours for $M$, which suggests the ability of the inverse power law model to sense cellular response more rapidly than the fractional modulation amplitude model. This method exhibits excellent stability in the control (0 μM Dox) data over a long time (6 days), suggesting the broad applicability of this technique for measuring functional cellular changes longitudinally.

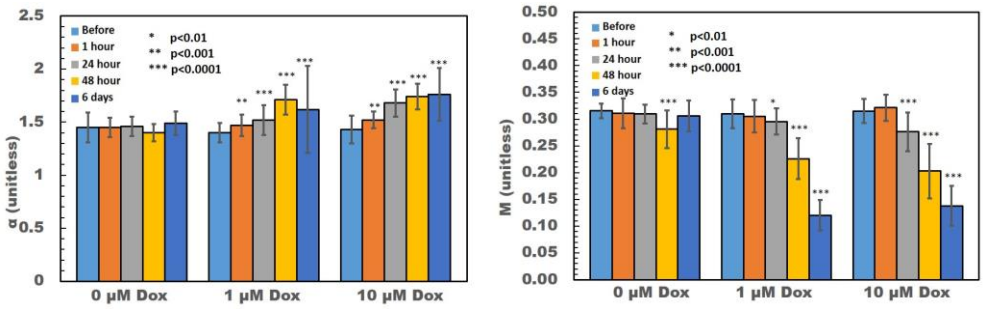


Figure 2. (Left) Results of $\alpha$; (Right) Results of $M$: time- and dose-dependent response of MEC organoids in 3D culture (MCF10DCIS.com) to doxorubicin (N=48-69 organoids per condition, mean ± standard deviation).

## 4. Conclusion

We proposed a quantitative tool for measuring motility of 3D breast cells in tissue cultures, and quantified the drug response of MEC organoids in 3D cultures as a function of time and dose. The metrics for quantifying cellular motility has been proven to be independent of SNR and image depth. This new platform will provide targetable metrics for assessing new treatments, and for studying tumor environmental factors driving metastasis. In the larger view, the development of quantitative, OCT-based motility imaging may be of broad importance to predict the action of anticancer drugs and tailor treatment decisions accordingly.

## 5. References

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