

## Quantifying Cellular Activity in Untagged Cells via Time-Lapse OIR Microscopy

Sylvie Landry\* and Werden J. Keeler\*\*

\* Thunder Bay Regional Research Institute, 980 Oliver Road, Thunder Bay, Ontario, Canada, P7B 6V4

\*\* Physics Dept., Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, Canada, P7B 5E1

Oblique incidence reflection (OIR) microscopy was used for the long-term optical monitoring of untagged live cells in cultures. Time-lapse movies and image analysis were used as complementary tools to visualize and quantify cellular activity from the many OIR images collected.

OIR microscopy employs highly oblique unfocussed episcopic illumination to produce bright images from light reflected and refracted from structural components in the sample. It is an ideal tool for gathering time-lapse information on cell dynamics with little photodamage and sample degradation. A time-lapse playback of image sequences shows the rapid movement of cellular components due to the metabolic activity in living healthy cells over a time span of several hours [1]. An image analysis program developed in-house provides a quantitative measure of the activity through the mathematical analysis of the variations in image intensity as a function of time. Two algorithms were implemented and described in [2]. Briefly, one algorithm computes the root-mean-square intensity (RMS I) associated with each image, and a second algorithm calculates the RMS value associated with the intensity difference computed on a pixel-by-pixel basis from adjacent images (RMS dI/dt).

Real-time monitoring and cell culturing techniques used in this study were as described in [1]. The cell line for which data are presented is a monolayer-adherent type culture of human origin. Two low-intensity voltage-regulated incandescent lamps were used to continuously illuminate the sample primarily with light having long wavelength in the visible and near-infrared range. A heated stage incubator and a 40x dry objective lens were used for time-lapse experiments. Image acquisition was controlled by software, and focusing of the specimen was performed manually.

Selected OIR images of two isolated cells used to demonstrate typical results of the computations are provided in Figure 1 and Figure 2. Both regions of interest were cropped from a field of view containing many bright cells distributed on a relatively uniform dark background. Results associated with a dark region for which no activity was recorded throughout the experiment are also provided for reference purposes. Figure 1 illustrates the time dependence of RMS I and RMS dI/dt associated with part of a cell crawling into and out of the cropped field of view. Figure 2 provides representative images illustrating the different stages of a cell undergoing mitosis. The overall image intensity (RMS I) increases to a maximum shortly after cytokinesis, and gradually decreases as the daughter cells move apart. The first peak in the derivative (RMS dI/dt) occurs where the cell rapidly collapses into a ball (around metaphase [3]). The intermediate well is due to the cell remaining in this quasi-stationary ball phase. The second peak occurs when the cell makes the transition from metaphase to cytokinesis. Other examples of cellular activity in small groups of untagged cells, such as macromolecular streaming and structural movement, are well described by this technique.

### References:

- [1] S. Landry et al., *Opt. Express* 12 (2004) 5754.
- [2] S. Landry et al., *Microsc. Microanal.* 13 (Suppl 2) (2007) 354CD.
- [3] E. Boucrot and T. Kirchhausen, *PNAS* 104 (2007) 7939.
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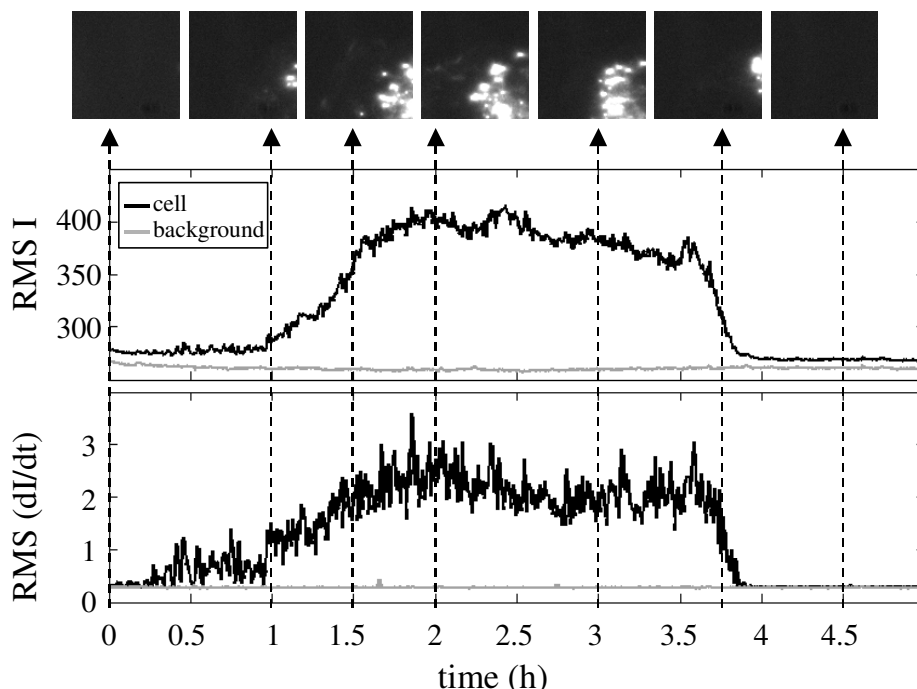


Fig. 1. OIR monitoring of an untagged SK-N-SH neuroblastoma cell crawling into and out of the field of view. RMS I provides a measure of the overall image intensity and RMS (dI/dt) provides a measure of the rate of change in cellular activity. OIR image frame width = 34 $\mu$ m.

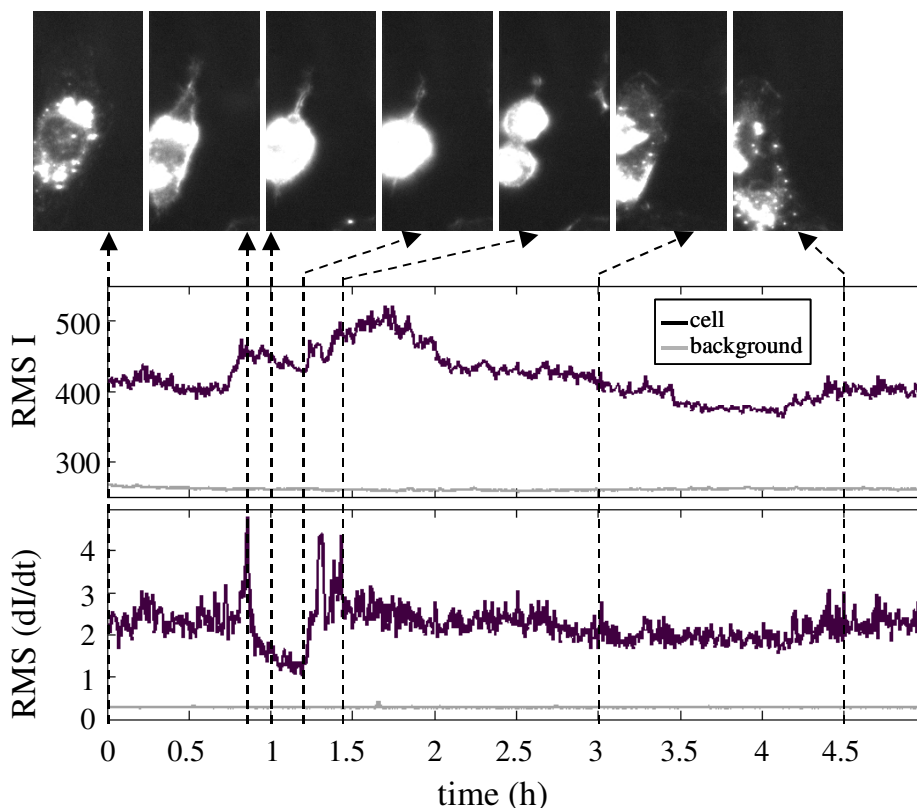


Fig. 2. OIR monitoring of an untagged SK-N-SH cell undergoing mitosis. As described in the text, the well shape structure in the RMS (dI/dt) curve is due to the cell rapidly collapsing into a ball around metaphase and then transitioning from metaphase to cytokinesis. OIR image frame width = 42 $\mu$ m.