ADAPTIVE ILLUMINATION CONTROL FOR LIVE CELL FLUORESCENCE MICROSCOPY

By

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Deleterious effects of high-intensity excitation light limit the number of images that can be recorded in live-cell fluorescence microscopy. This thesis presents the development, implementation, results, and analysis of a technology which avoids collecting unnecessary out-of-focus images by restricting image acquisition to small dynamic regions centered on objects of interest (OOI). This approach, which is termed Adaptive Illumination Control for Live-cell Fluorescence Microscopy (AIFM), utilizes computer vision to detect and track OOIs at time point t and a statistical motion model to form a range sufficient to image OOIs time point $t + 1$. The technology was developed and implemented on a standard widefield inverted fluorescence microscope.

AIFM reduces the total light exposure by limiting image acquisition to the focal planes immediately adjacent to the position of the OOI. With traditional time-lapse imaging techniques, a fixed acquisition region leaves room for cells to move and change shape. This results in a large number of unnecessary exposures which damage cells and affect experiment outcomes. AIFM allows a biologist to select features of interest, creating a dynamic acquisition region which moves with the cell or organelles of interest. This allows for a higher sampling resolution and a reduction in photodamage.

In experiments with 25 conventionally illuminated cells and 21 AIFM illuminated cells, 81% of the AIFM illuminated cells progressed through mitosis normally (18-30 minutes). The remaining 19% of cells experienced delayed mitosis, taking 37-52 minutes to divide. Of the 25 conventionally imaged cells 28% progressed through mitosis normally, 20% experienced delayed mitosis, and 52% arrested in mitosis and did not divide.

With the widespread use of fluorescent proteins, photobleaching, or deterioration of these proteins due to light exposure, is of serious concern and research interest. AIFM reduces photobleaching when compared to conventionally illuminated cells imaged with the same parameters. The averaged normalized signal strength of the centrosomes of 5 AIFM imaged cells was 83% after one hour. The same conditions were used to image 5 cells conventionally and the averaged normalized signal strength was 51% after one hour.

The novelty of this approach arises from the connection of a computer vision based object detection and tracking algorithm to the illumination source and stage controls of a live cell fluorescence microscope. Control of the illumination source based on detection and tracking data allows for the previously unattainable goal of long duration live cell fluorescence microscopy and provides live-cell fluorescent microscopy experiments with longer lasting fluorescent protein tags, healthier cells, and higher quality images. Additionally, tracking the objects in real time results in a complete motion history of the OOI's, eliminating the need for post processing to obtain the OOI motion history.