

AUTOMATIC REAL-TIME DETECTION OF THE ONSET OF APOPTOSIS IN CONFOCAL FLUORESCENCE MICROSCOPY

P Paul¹, D Kalamatianos¹, H Huber², J Wenus², H Düßmann²

¹ Hamiton Institute, National University of Ireland, Maynooth, Ireland
{perrine.paul, dimitris.kalamatianos}@nuim.ie

² Department of Physiology and Medical Physics, RCSI, Dublin 2, Ireland
{heinhuber, jakubwenus, hduessmann}@rcsi.ie

KEYWORDS: Live Cell Imaging, Image Processing, Apoptosis, DIC

One of the main challenges in fluorescence imaging of live cells when studying apoptosis is to protect the cells from photodamage in order not to interfere with the experiment. It was shown in [1] that the mitochondria membrane potential depolarization could be measured via the intensity of TMRM fluorescence (Tetramethyl Rhodamine Methyl Ester) and used as the temporal reference point for all other apoptotic signalling events. But the lack of real time image processing tools is a limiting factor for an objective detection of the onset of apoptosis. A fully automatic tracking algorithm was developed allowing automatic detection of the onset of apoptosis in real time by monitoring the TMRM average pixel intensity per cell against a reference value computed online on the first records for each cell individually [2]. These cells were initially segmented as described in [3]. In order to prevent any unnecessary phototoxicity, this algorithm uses only the DIC (Differential Interference Contrast) channel to estimate the shape and location for each cell in less than 0.1 second per cell, using a 2.33 GHz processor with 2 GB of RAM. An example of results generated online without any human intervention after the set up of the experiment is presented in Figure 1.

The combination of this method and FRET for example could extend the number of observable apoptotic events and allow their detection with higher precision as stated in [1].

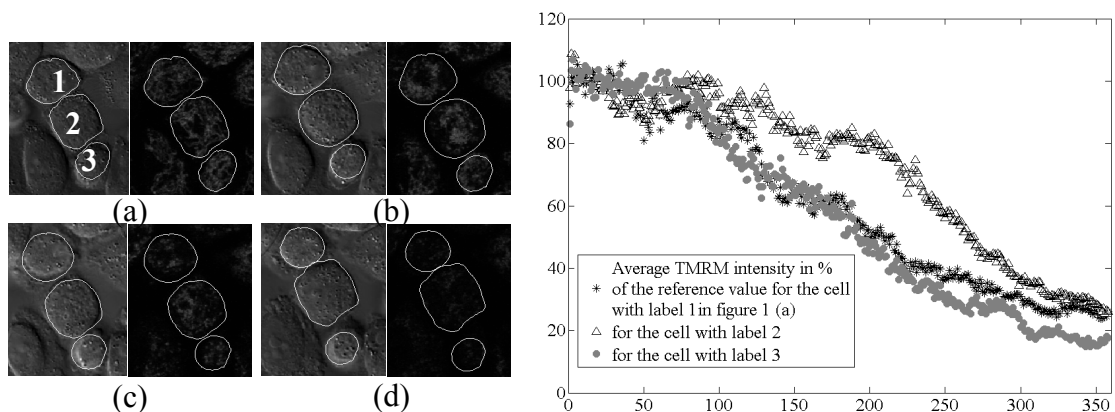


Figure 1: (a)-(d): Cell shapes automatically tracked using the DIC channel only, superposed to the DIC and TMRM channel for records 2, 100, 200 and 300 respectively. (e) TMRM average pixel intensity relative to the reference value computed on the first 30 records, against the record number for the 3 cells shown in (a)-(d).

REFERENCES:

- [1] H. Düßmann, M. Rehm and J.H.M. Prehn. "Focus on Apoptotic Signalling in Living Cells", *Focus on Microscopy*, p77, Jena, Germany (2005)
- [2] H. Düßmann, M. Rehm, D. Kögel and J.H.M. Prehn "Outer mitochondrial membrane permeabilization during apoptosis triggers caspase-independent mitochondrial and caspase-dependent plasma membrane potential depolarization: a single-cell analysis". *J Cell Sci.* 1;116(Pt 3):525-36. (2003)
- [3] P. Paul, D. Kalamatianos, H. Düßmann and H. Huber "Automated live cell image segmentation for on-line high-content screening", *ICSB*, p181, Gothenburg, Sweden (2008)