PHOTOSTIMULATION OF CA^{2+} TRANSIENTS IN LIVE CELLS

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The modulation of intracellular Ca^{2+} plays a large role in controlling key cell functions such as cell division, signaling, contraction and cell death. To date, intracellular Ca^{2+} dynamics have mainly been investigated using electrophysiological measurement techniques such as patch clamp experiments often used in combination with fluorescence imaging techniques. To activate intracellular channels, drugs are often used. However, diffusion and mixing constraints provide little control over the time course of channel activation and deactivation. In addition, the invasive nature of conventional whole cell patch clamp techniques perturbs the intracellular environment and can alter channel behaviour.

We report a non-invasive technique that induces controlled Ca^{2+} responses in isolated smooth muscle cells and cardiac myocytes using an LED light source as a stimulus. Using a conventional epi-fluorescence microscope configuration, cells labeled with a Ca^{2+} fluorescent indicator (Fluo-3AM) were stimulated using the low intensity light (<1.5mW @ λ=488nm) and the resultant Ca^{2+} transients were visualized using a highly sensitive CCD camera. A typical fluorescence plot of a light induced Ca^{2+} transient in a smooth muscle cell is shown in Fig.1.a. alongside the associated images of four consecutive timepoints within the duration of the Ca^{2+} transient in Fig.1.b.

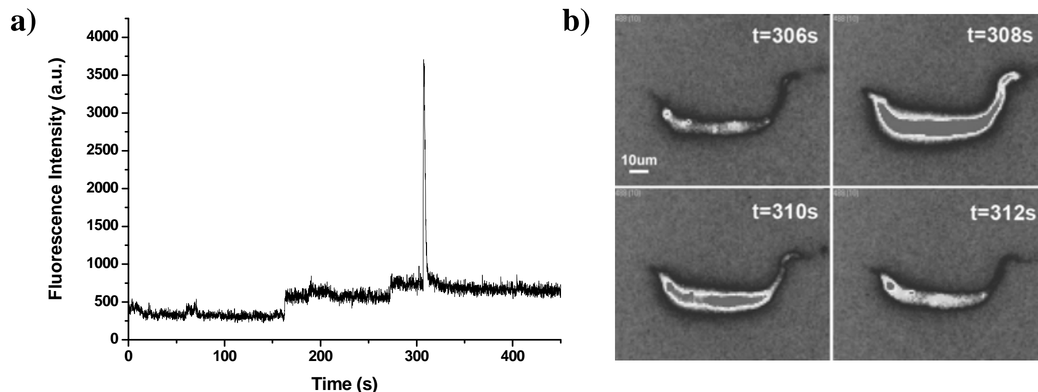

Figure 1: a) A typical plot of a light induced Ca^{2+} transient in a smooth muscle cell. b) Fluorescence images of four consecutive timepoints within the duration of the Ca^{2+} transient shown in a.

We will describe the cell stimulation protocol used and present data demonstrating the efficacy of this low cost and minimally invasive technique. We will also describe the investigation into the origin of the light induced Ca^{2+} responses.