A 340/380 nm LED illuminator for Fura-2 ratiometric Ca^{2+} imaging of live cells with better than 5 nM precision

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Introduction

Cytosolic Ca^{2+} plays an integral role in cells and the study of its dynamics can reveal much about biological processes [1]. Fura-2 can provide quantitative data on cytosolic Ca^{2+} changes by exciting at 340 nm and 380 nm and taking the ratio of the emission at both wavelengths [2].

Traditionally for this type of imaging an arc lamp had to be used for illumination as LEDs of the appropriate wavelengths were not available [3]. LEDs hold advantages over arc lamps by exhibiting high amplitude stability and the ability to rapidly switch between wavelengths.

We aimed to test a new 340/380 nm LED system for use in ratiometric Fura-2 AM Ca^{2+} imaging and present results using tsA-201 cells and hippocampal neurons.

Methods

- Specimens were washed three times with HEPES-buffered saline solution and loaded with Fura-2 AM for 60 minute at 37 °C. They were then washed a further three times before imaging.
- **HBS Control solution: -** 1 litre of distilled water containing (in mM): NaCl, 140; KCl, 5: $MgCl_2$, 2; HEPES, 10; D-glucose, 10: $CaCl_2$, 2 at a pH of 7.4.
- **Power at the specimen plane: - 340 nm:** 1.35 mW
  380 nm: 1.40 – 3.08 mW
- **LED exposure time: - 0.5 Hz imaging:** 100 ms
  24.39 Hz video-rate imaging: 20.5 ms
- **Emission detection:-** Hamamatsu ORCA-Flash 4.0 CMOS camera with a binning n = 2

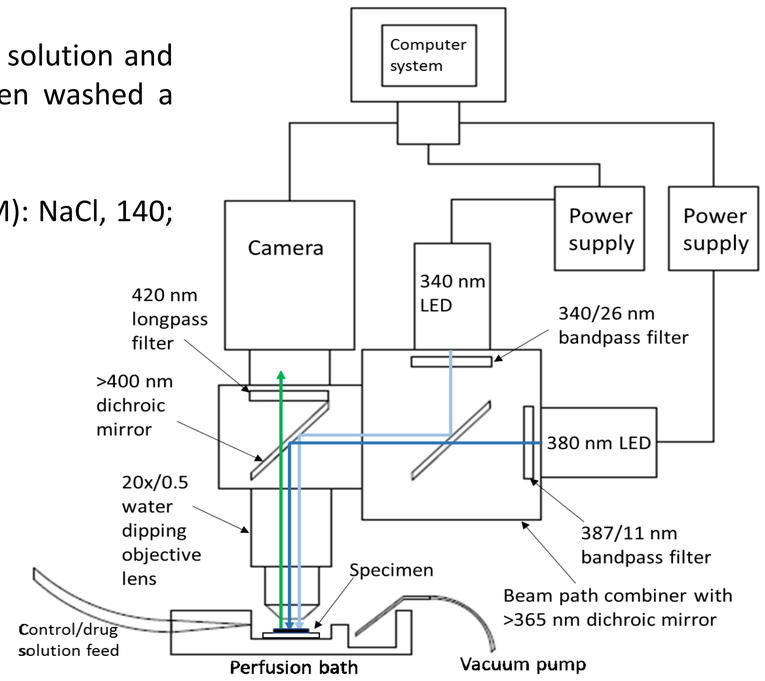

Figure 1: Schematic diagram of Olympus BX50 microscope and imaging apparatus

0.5 Hz ratiometric Fura-2 AM Ca^{2+} imaging of drug-mediated responses in tsA-201 cells and hippocampal neurons

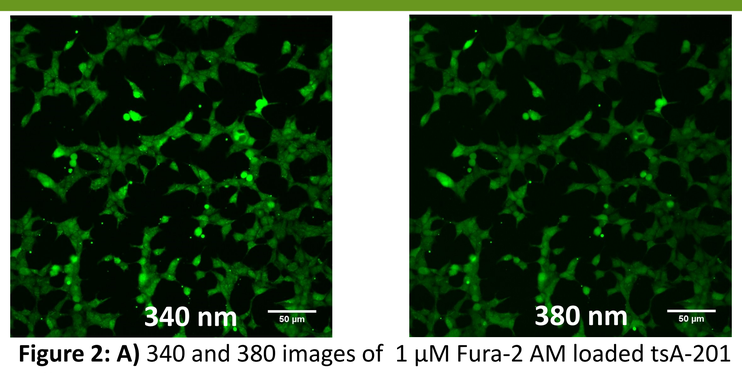

Figure 2: A) 340 and 380 images of 1 µM Fura-2 AM loaded tsA-201 cells

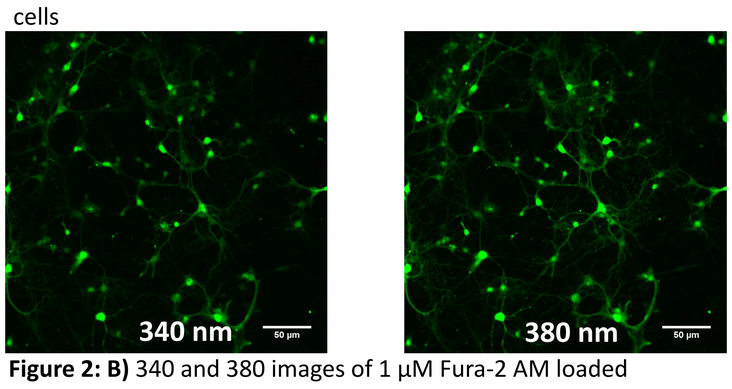

Figure 2: B) 340 and 380 images of 1 µM Fura-2 AM loaded hippocampal neurons

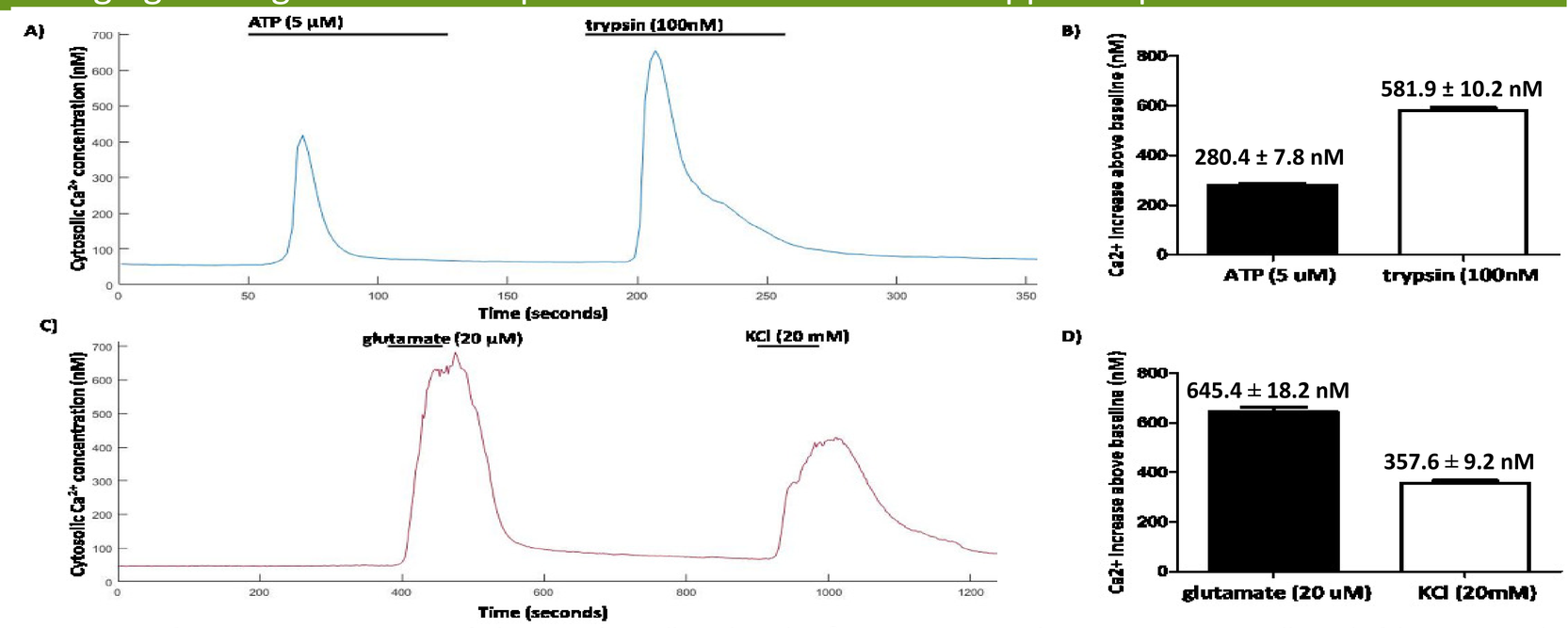

Figure 3: A) Representative traces of pharmacologically induced Ca^{2+} concentration changes in tsA-201 cells and **C)** hippocampal neurons. **B)** Average Ca^{2+} increase for each stimuli in tsA-201 cells (n = 572) and **D)** hippocampal neuron (n = 388).

Ca $^{2+}$increases are in agreement with previous experiments in the same cells illuminated by arc lamps [4 - 8]

Average fluctuations in basal Ca^{2+} levels

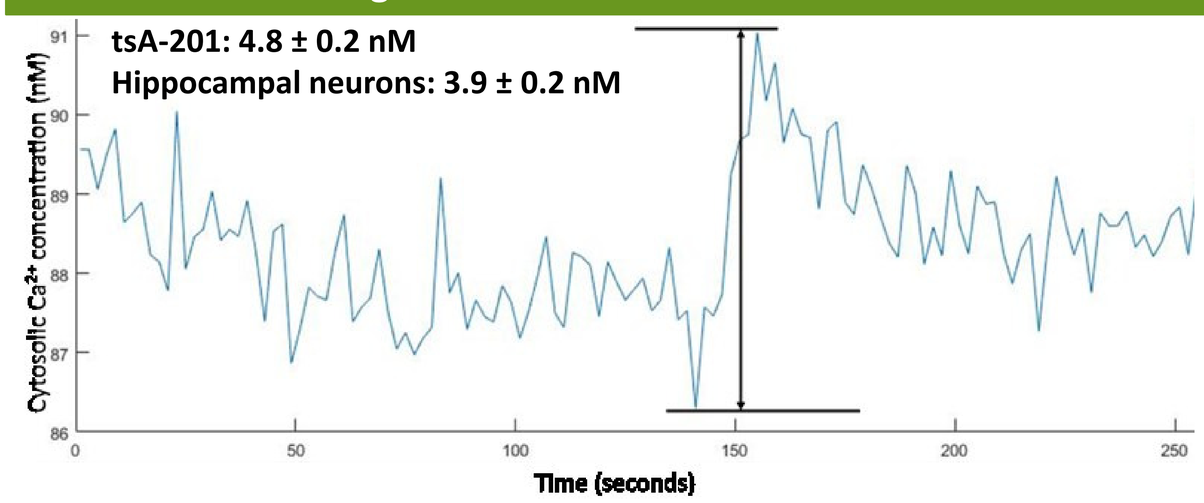

Figure 4: Example plot of baseline Ca^{2+} fluctuations in hippocampal neurons

The fluctuations are below the theoretical 5 – 10 nM precision of Fura-2 [9]

Ca^{2+} imaging of tsA-201s loaded with lower concentrations of Fura-2AM

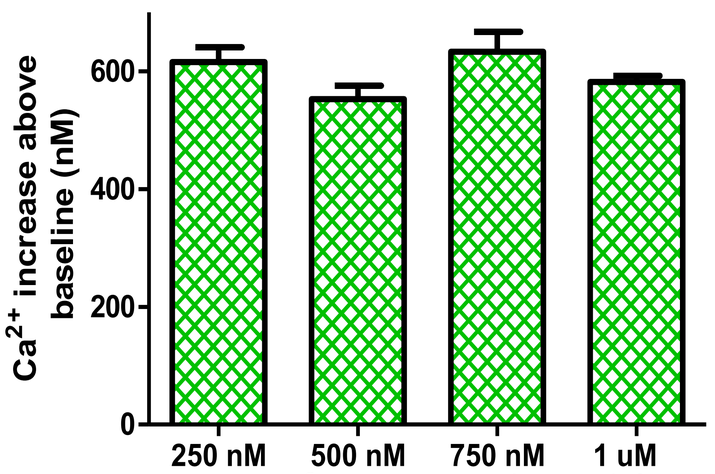

Figure 5: Ca^{2+} responses obtained from tsA-201 cells loaded with different concentrations of Fura-2 AM

No significant change in obtained trypsin (100 nM) Ca^{2+} response

Advantages of using lower concentrations:

- Increasing the number of uses from the vial
  – **Reduced cost**
- Improving cell viability
  – **Cells live longer**

Fura-2 ratiometric imaging of synaptically-driven Ca^{2+} events in hippocampal neurons

Figure 6: Synaptically-driven Ca^{2+} events captured at a rate of **A)** 0.5 Hz in a single neuron and **B)** 24.39 Hz in two neurons

Conclusions

- **This is the first application of a truly 340/380 nm LED illuminator for Fura-2 ratiometric Ca^{2+} imaging of live cell specimens with a precision that is only limited by the response of Fura-2.**
- **Using this illuminator it is now possible to use Fura-2 AM dye concentrations as low as 250 nM offering both an economical and cell viability advantage.**
- **Video rate imaging of synaptically-driven Ca^{2+} events combines high temporal and spatial resolution to obtain higher throughput information than previously possible [10].**
- **This LED illuminator combines optimum excitation and high stability wavelength switching to free Fura-2 imaging from illumination problems experienced in the past.**

References:[1] Berridge, M. J., *et al.,* Nat. Rev. Mol. Cell Biol (2000), 11–21**(1), [2]** Grynkiewicz, G. *et al.,* J. Biol. Chem. (1985), 3440 – 3450 **(260), [3]** Bootman, M. D. *et al.,* Cold Spring Harbour Protocol (2013), **[4]** Wu, J. et al., Biochem J. (2014), 13–22 **(464)**, **[5]** Aulestia, F. J. et al., Biochem. J. (2011), 227–35 **(435) [6]** Verderio, C. *et al.,* Proc. Natl. Acad. Sci. U. S. A. (1995),6449–6453 **(92), [7]** Poole, D. P. *et al.,* J. Biol. Chem. (2013), 5790–5802 **(288), [8]** Jung, S. R. *et al.,*J. Gen. Physiol. (2016), 255 **(147), [9]** Petersen, O., *Measuring Calcium and Calmodulin Inside and Outside Cells* (2013), **[10]** Scanziani M. *et al.,* Nature (2009), 930–939 **(461)**