NeuO and CDR3: fluorescent probes labeling neurons and neural stem cells from mouse subventricular zone: widefield microscopy combined with long-term live cell imaging facilitated by microfluidic perfusion and fluorescent dye reloading

Michael D. Lovelace[1-3], Varda S. Sardesai[1], Michael W. Weible II[4], Bruce J. Brew[1,2,5]
Email: m.lovelace@amr.org.au

1 Applied Neurosciences Program, Peter Duncan Neurosciences Research Unit, St Vincent's Centre for Applied Medical Research, Sydney, New South Wales, Australia
2 Faculty of Medicine, St Vincent's Clinical School, University of New South Wales Sydney, New South Wales, Australia
3 Discipline of Anatomy and Histology, School of Medical Sciences, The University of Sydney, Sydney, New South Wales, Australia
4 School of Natural Sciences, Griffith University, Brisbane, Queensland, Australia
5 Department of Neurology, St Vincent's Hospital, Sydney, New South Wales, Australia

KEYWORDS: Widefield microscopy, microfluidic perfusion, neural stem cells, neurons, dye

Genetic approaches to label brain cells can suffer from poor transfection efficiency. Alternatives include dyes loaded into cells to identify specific cell types, however these are typically depleted from cells over several hours via cell surface transport pumps and thus accumulate in the extracellular media. This limits their use to short term imaging only. Dye fluorescence is also compromised by photobleaching, particularly on widefield microscopy. Microfluidic perfusion system technologies have rapidly progressed recently, and enable pre-programming of perfusion parameters (solution flow speed, time etc), and switching different solutions. This saves time, lowers reagent use due to smaller volumes and enhances data reproducibility. To date, a system of reloading dyes into cells, enabling sustained imaging and tracking of cell types over many hours using microfluidic perfusion has not been explored.

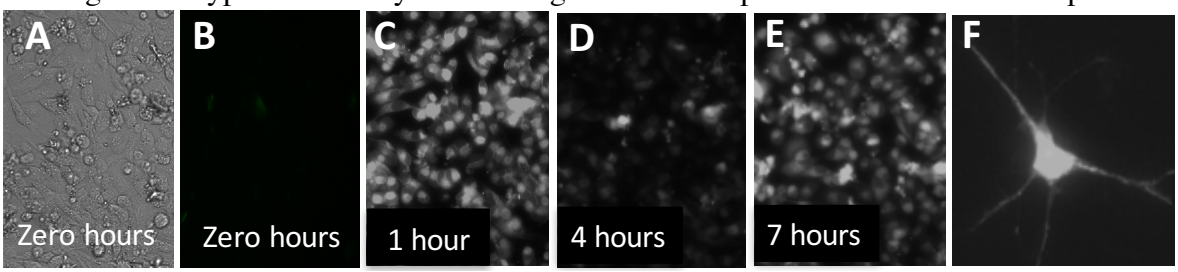

Fig. 1: (A) DIC. **(B)** Green channel; no dye loaded. **(C)** 1hr; NeuO loaded. **(D)** 4hrs; cells excrete dye so are dimmer **(E)** 7 hrs; same cells reloaded. **(F)** Single NeuO-labelled neuron.

Recently, fluorescent celltype-specific dyes NeuO (for neurons) and CDR3 (for neural progenitors) have been developed[1-2]. Cultured neurons and undifferentiated neural precursors derived from mouse subventricular zone can be loaded with dyes (within 40-60 minutes) but as expected they are progressively depleted from cells in static culture hours after loading. We show that repeated reloading of cells with fresh dye using a commercial Cellasic ONIX 2 microfluidic system is possible without compromising cell viability (**Fig. 1**). This enables longer live cell imaging (e.g. overnight) than otherwise possible, and in turn, tracking of single cells, drug responses etc. NeuO and CDR3 are spectrally apart, allowing simultaneous imaging, and antibodies to cell surface markers can be co-mingled. Experimental approaches and plate fluidics regimes will be shown. In summary, we show a method of cell labelling for live cell imaging that reduces labour and reagent costs while enabling longer imaging.

References: [1]. Er, J. C., Leong, C et. al. "NeuO: a fluorescent chemical probe for live neuron labeling" *Angewandte Chemie* **54**, 2442–2446 (2015). [2]. Leong, C., Zhai, D. et. al "Neural stem cell isolation from the whole mouse brain using the novel FABP7-binding fluorescent dye, CDr3" *Stem cell research* **11**, 1314–1322 (2013).