DIFFERENTIAL STABILITY OF GFP AND YFP IN FRUIT CLEARED BRAINS

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Background Green Fluorescent Protein (GFP) and its genetic mutant form - Yellow Fluorescent Protein (YFP) have given rise to a vast number of transgenic reporter animals. The rapid development of tissue clearing techniques demands reporter models expressing stable fluorescent markers under different clearing media. There have been reports regarding the effects of the molecular environment and pH on GFP stability [1]. We hypothesized that GFP signals, contrary to YFP, decline over time in brains from reporter mice when cleared with the FRUIT protocol.

Methods Brains from Cx3Cr1-GFP and Thy1-YFP reporter mice were fixed in 4% PFA and sectioned in 3 mm thick slices before clearing with the FRUIT protocol [2]. Cleared thick slices were imaged under a two-photon laser scanning microscope (Leica SP5, 20x/1.00 water HCX PL APO L, WD 2mm, λ_{exc} 820 nm) at day 0 (final day clearing protocol) and at day 5. Z-stacks (max thickness 1.3mm; z steps 1.98µm) were analyzed with ImageJ. Total cell intensity (INTtotal) and noise (INTnoise) were quantified from 3-10 cells per FOV every 100µm. GFP signals from microglia cells (Cx3Cr1-GFP) and YFP signals from neuronal cells (Thy1-YFP) were calculated as follow: INTcell=INTtotal-INTnoise. Signal-to-Noise ratio (SNR) was calculated as INTcell:INTnoise.

Results SNR was impacted by time and stack depth. Further analysis of INTcell of microglia and neuronal soma revealed GFP signal decreased between d0 and d5 while YFP signal was maintained [Fig. 1]. In addition, INTnoise for GFP, but not YFP, was increased over time on the sample surface.

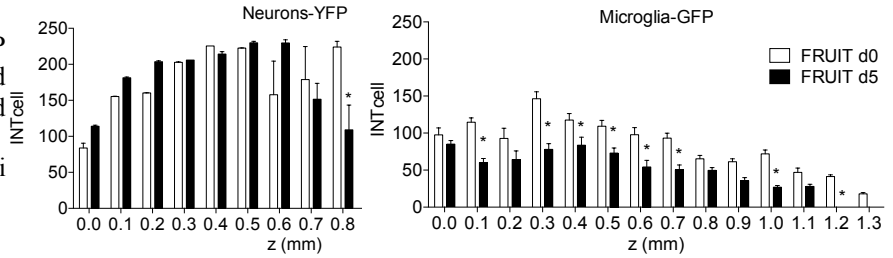

Figure 1: YFP (left) and GFP (right) signals from neurons and microglia respectively at d0 and d5 after FRUIT clearing.
Two way ANOVA, Bonferoni post-test: * $p<0.05$ vs FRUIT d0.

Conclusion GFP signal from microglia cells decreases over time in FRUIT cleared samples while YFP signal from neuronal cell was maintained. This should be taken into account while clearing samples containing GFP signals with FRUIT, as this may lead to under estimation of GFP cells, an impaired storage and limited imaging opportunities in time. Potential explanations to be explored regarding this FRUIT-induced GFP signal decline are the influence of pH (pH_{FRUIT} [8.2-8.7]) on GFP as well as sample oxidation at the surface.

References

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