FAST TIME-GATED FLIM ENABLES HIGH-THROUGHPUT FUNCTIONAL IMAGING OF PROTEIN AGGREGATION IN MOVING *C. elegans*

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ABSTRACT: The nematode worm *Caenorhabditis elegans* is an important model organism to study the molecular mechanisms of protein misfolding diseases associated with amyloid formation. However, obtaining a reliable, quantitative read-out of protein aggregation in this model system remains a challenge. To address this problem, we present a time-gated fluorescence lifetime imaging (TG-FLIM) method [1] that captures fluorescence decay dynamics faster than conventional systems. This allows us to gain functional insights into the protein aggregation process in living animals by enabling rapid characterisation of the level of aggregation of amyloid proteins. In longitudinal studies of *C. elegans* models, we observed marked differences in the aggregation kinetics and the nature of protein inclusions formed by α-synuclein and polyglutamine (proteins associated with Parkinson's and Huntington's diseases, respectively). In particular, we found that α-synuclein aggregates develop amyloid-like features only in aged worms, whereas polyglutamine forms amyloid characteristics rapidly in early adulthood. Furthermore, we show that the TG-FLIM method is capable of imaging live worms moving in specially designed agarose micro-chambers, using a 'digital worm stretching' algorithm that also corrects for motion artefacts in the FLIM signal. Taken together, our results show that the TG-FLIM method enables high-throughput functional imaging of living *C. elegans*. It can therefore be used to study *in vivo* mechanisms of aggregation and could aid the search for therapeutic modifiers of protein aggregation and toxicity.

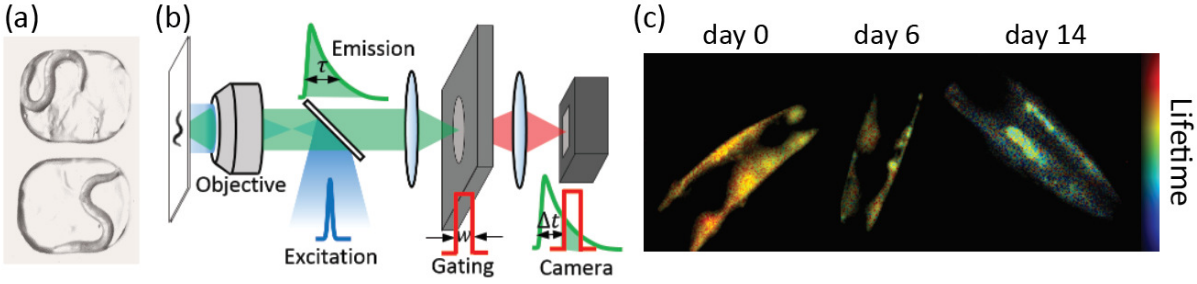

Figure 1: (a) *C. elegans* in specialized micro-chambers. (b) Schematic of the TG-FLIM setup, which uses shifting temporal gates to capture the fluorescence decay. (c) Longitudinal study of α-synuclein aggregation across the lifespan of the model worm as evidenced by a decrease in fluorescence lifetime.

[1] Laine, R. F. *et al.* "Fast fluorescence lifetime imaging reveals the maturation process of α -synuclein aggregates in ageing Caenorhabditis elegans." *bioRxiv* **414714,** 1–18 (2018).