Supporting information related to:

Fast fluorescence lifetime imaging reveals the aggregation processes of α-synuclein and polyglutamine in aging *Caenorhabditis elegans*

Romain F. Laine[1,a,*], Tessa Sinnige[2,*], Kai Yu Ma[2,b], Amanda J. Haack[3,c], Chetan Poudel[1], Peter Gaida[1,d], Nathan Curry[1,e], Michele Perni[2], Ellen A.A. Nollen[4], Christopher M. Dobson[2], Michele Vendruscolo[2], Gabriele S. Kaminski Schierle[3$], Clemens F. Kaminski[1$]

[1]*Laser Analytics Group, Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB3 0AS, UK*

[2]*Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK*

[3]*Molecular Neuroscience Group, Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB3 0AS, UK*

[4]*European Research Institute for the Biology of Aging, University Medical Centre Groningen, 9700 AD Groningen, The Netherlands*

[$]*Correspondence: gsk20@cam.ac.uk and cfk23@cam.ac.uk*

** Equal contribution*

[a]*Current address: Medical Research Council Laboratory for Molecular Cell Biology (LMCB), University College London, United Kingdom*

[b]*Current address: Neurogenetics Group, Department of Genetics, University Medical Centre Groningen, The Netherlands*

[c]*Current address: Medical Scientist Training Program, University of Washington, Seattle WA, USA*

[d]*Current address: Ostbayerischen Technischen Hochschule (OTH) Regensburg, Germany*

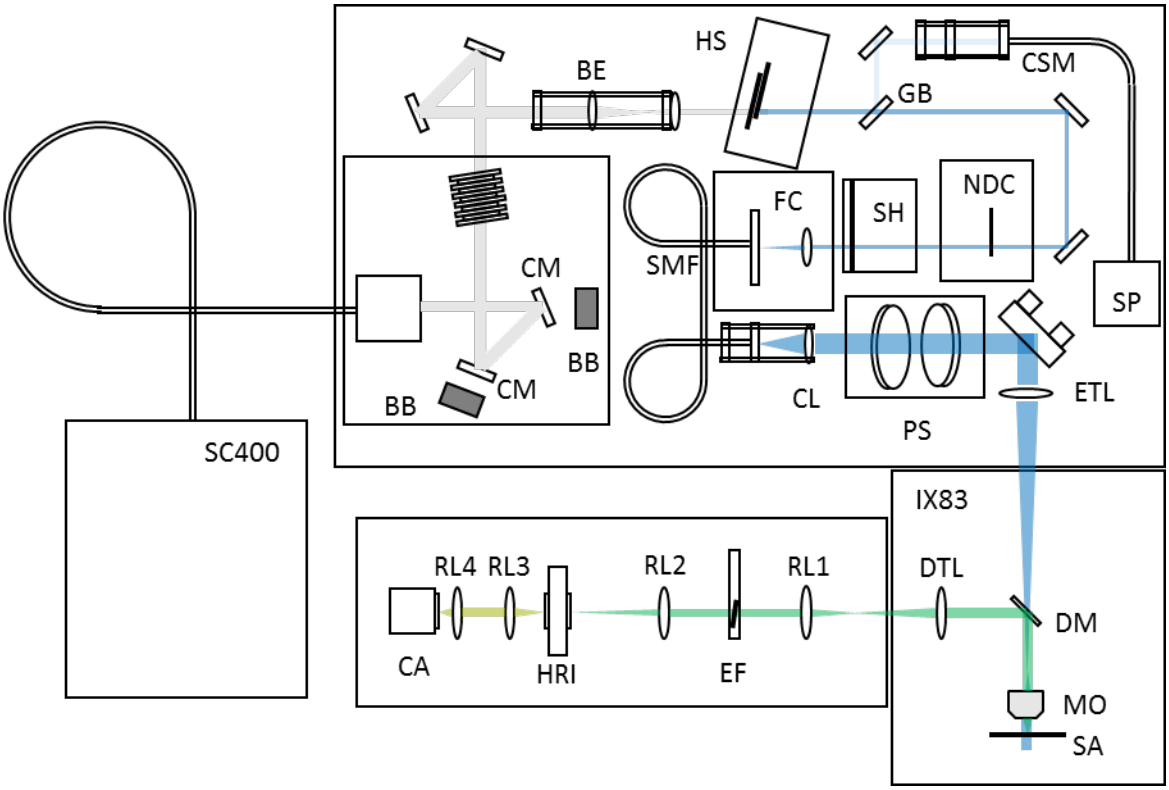
[e]*Current address: Photonics Group, Department of Physics, Imperial College London, United Kingdom*

Details of TG-FLIM microscope architecture

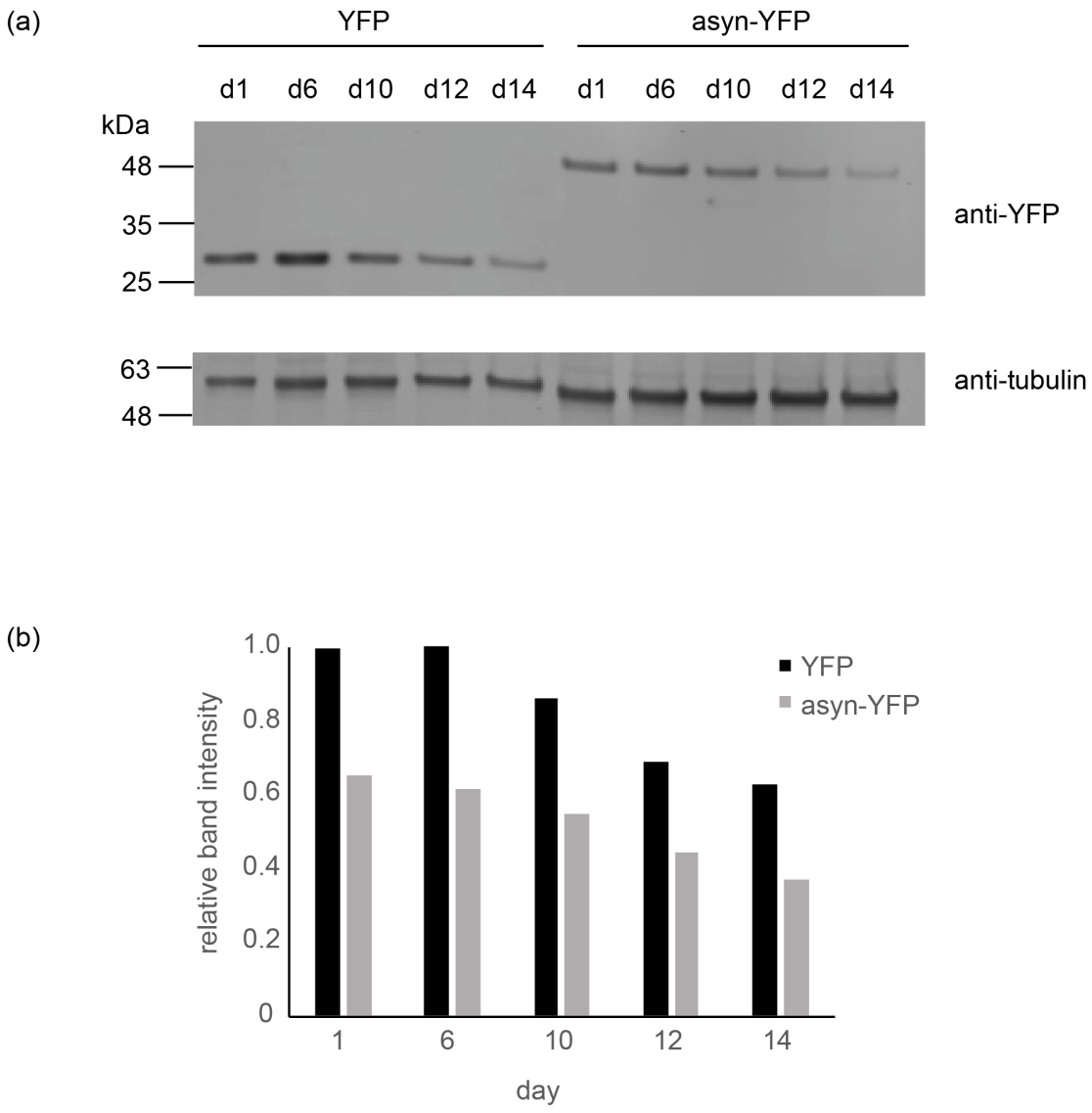
With TG-FLIM technology, the fluorescence decay of each pixel in the image is obtained by temporally gating the fluorescence signal reaching the camera using a High-Rate Imager (HRI). During the camera exposure (in the millisecond range), only the fluorescence originating from a precisely set temporal window with respect to the excitation pulses of the laser (in the nanosecond range) is allowed to reach the camera. For instance, a single 50 ms camera exposure, 40 MHz laser repetition rate and a 1 ns HRI gate width will lead to an accumulation of fluorescence signal on the camera originating from 2 millions temporal gates, corresponding to a total of 2 ms of temporally selected fluorescence signal. The relative position of the temporal gate can then be accurately shifted in time with picosecond precision. Therefore, a sequential acquisition of camera frames at different gate positions can sample the fluorescence decay at the nanosecond scale and allow for the measurement of fluorescence lifetime in all pixels in the image.

The optical layout of the TG-FLIM is shown in **Supplementary Figure 1**. The laser source was provided by a super-continuum laser (Fianium, SC400-4). The Hof stage performs the spectral selection of the excitation beam via a combination of long-pass and short-pass linear variable filters (Delta Optics, Germany). The selection is calibrated but can be checked on a spectrophotometer (Qwave, RGB photonics, Germany). The neutral density control (linear variable neutral density) allows a control of the power reaching the sample. All components were obtained from Thorlabs. The excitation beam is launched into an automated Olympus IX83 frame equipped with an automated stage (Prior). The image formed after the detection tube lens of the frame is relayed onto the photocathode of the HRI (HRI Gen 2 18mm, Kentech Instruments, Ltd., UK) and the fluorescence is spectrally selected by a fluorescence filter (Prior filter wheel) place in the Fourier plane of this optical relay. The image of the sample formed on the phosphor screen of the HRI is then optically relayed onto the camera (PCO USB pco.pixelfly) using a combinations of two SLR lenses.

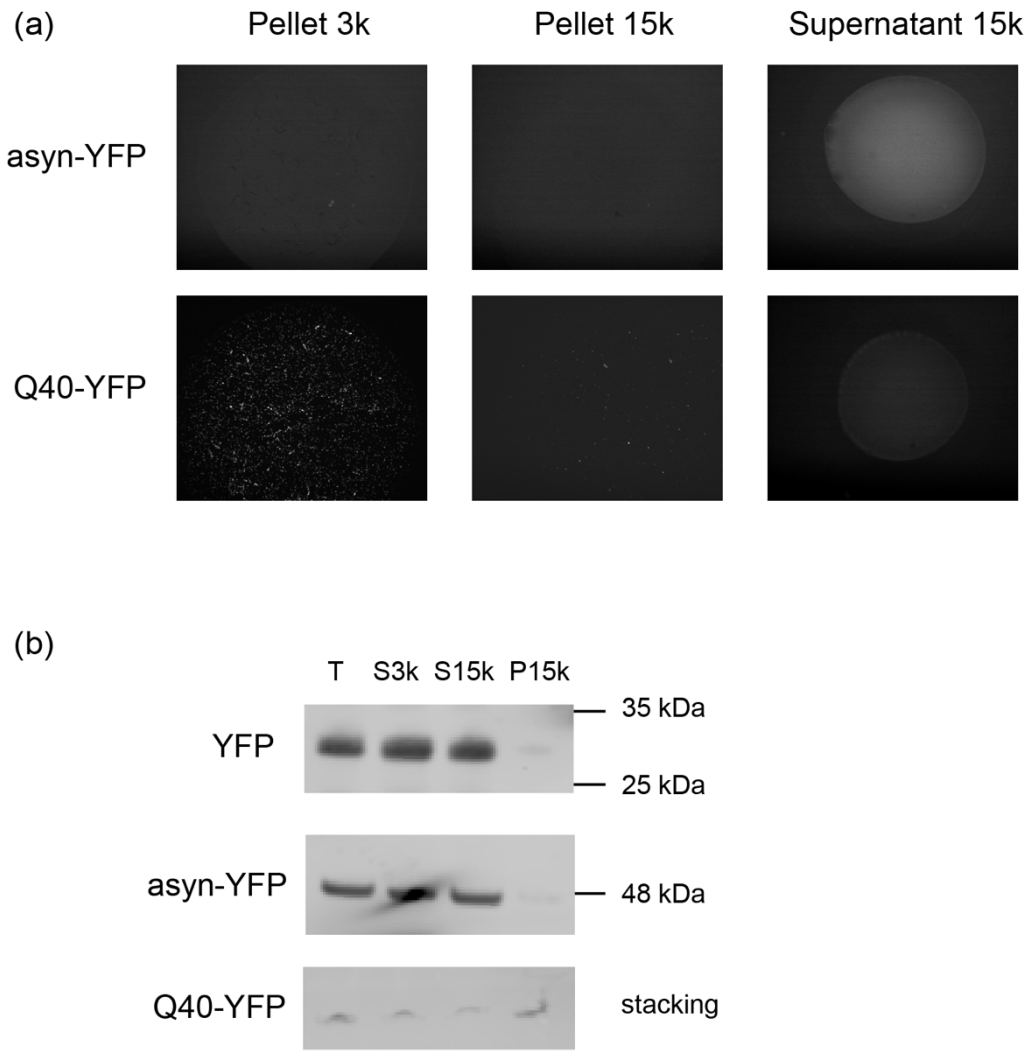
The electronic delay between the excitation pulse and the detection gating was controlled by two consecutive precision delay generators (Kentech Instruments, Ltd., UK). One was used to adjust the maximum intensity of the decay at 2 ns delay of the second delay generator. The second delay generator was used for scanning the delays between within the measurement and was externally triggered by the camera exposure signal from the camera, which was set as free-running.



Supplementary Figure 1. Optical layout of the TG-FLIM microscope. SC: super-continuum source. BB: beam block. CM: cold mirror. BE: beam expander (collimator). HS: Hof stage. GB: glass block (5% reflection). CSM: coupling to spectrometer. NDC: Neutral density control. SH: shutter. FC: fibre coupling. SMF: Single-mode fibre. CL: collimating lens. PS: periscope. ETL: Excitation tube lens. DM: Dichroic mirror. MO: Microscope objective. SA: Sample. DTL: Detection tube lens. RL: Relay lens. EF: Emission filter. HRI: High-rate imager. CA: Camera.



Supplementary Figure 2. Expression levels of YFP and α-syn during ageing of the nematodes. (a) Western blot of YFP and α-syn worms probed with anti-YFP antibody. Worms were picked into denaturing buffer at the indicated days of adulthood and equivalent numbers were loaded for each time point. The same blot was probed with anti-tubulin as a loading control. (b) Quantification of the blot shown in (a). Band intensities of YFP and α-syn were divided by the tubulin band intensities and normalised to YFP at day 1.

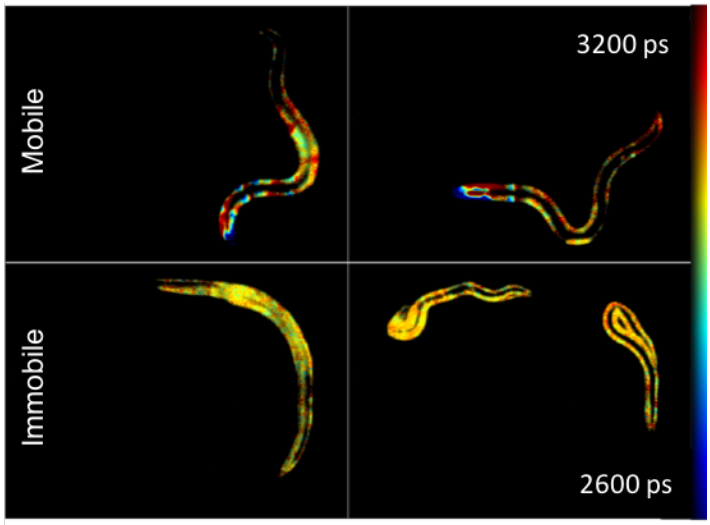


Supplementary Figure 3. Analysis of worm lysates prepared at day 6 of adulthood. (a) Fluorescence microscopy of fractionated worm lysates in detergent-containing buffer. Pellets after low speed centrifugation (3,000 rpm), high speed centrifugation (15,000 rpm) and the final supernatant are shown. α-syn-YFP fluorescence was only observed in the soluble fraction and no longer localised to inclusions, whereas Q40-YFP inclusions persisted and were exclusively present in the pellet fractions. (b) Western blot of YFP, α-syn and Q40 lysate fractions probed with an anti-YFP antibody. YFP and α-syn are predominantly present in the soluble fractions, whereas Q40 was observed in the pellet. Note that the Q40 inclusions do not enter the gel efficiently, and signal was detected on the border of the stacking gel.

Live C. elegans in agarose micro-chamber

Supplementary Figure 4. Living *C. elegans* in agarose micro-chamber.

https://youtu.be/Ws6w4BVbrkM



Supplementary Figure 5. Motion artefacts on the fluorescence lifetime images. The animals on the top have been moving during the acquisition whereas the ones at the bottom remained immobile during the acquisition. Large “rainbow” artefacts are visible especially around the head of the animal.