Code used for diameter measures as in Korte, Barkaway, Wells, Freitas, Sethi, Andrews, Skidmore, Stevens, Attwell (2024) Nature Neuroscience.

We would like to thank John S.Y. Lim (A\*STAR Microscopy Platform, Singapore) for providing code that computes the full width at half maximum (FWHM) of a Gaussian fit to the fluorescence intensity profile of a line selection in Fiji (ImageJ). The code was modified by Korte et al., to measure the full width at quarter maximum (FWQM), and to measure the FWHM or FWQM from a line selection for multiple frames in a timeseries recording (movie).

Save the required macro file on your computer:

FWHM\_SingleFrame.ijm

Computes the FWHM from a line selection in a single-frame image.

FWHM\_MultipleFrames.ijm

Computes the FWHM from a line selection for multiple frames in a timeseries recording (movie).

FWQM\_SingleFrame.ijm

Computes the FWQM from a line selection in a single-frame image.

FWQM\_MultipleFrames.ijm

Computes the FWQM from a line selection for multiple frames in a timeseries recording (movie).

Open the image or movie in Fiji.

For single-frame images or z-stacks, select the channel required for the measurement (if the image has multiple channels). Select the straight line tool and draw a line over the object for which the diameter measurement is required. Adjust the line width as required. Drag and drop the 'FWHM\_SingleFrame.ijm' or 'FWQM\_SingleFrame.ijm' macro into Fiji and press 'Run'. A ‘Warning: Poor Fitting’ message will appear if r2 is <0.6 for the Gaussian fit. The line name, the FWHM or FWQM value and unit (e.g. μm) will appear in a table. To save the line selection, select 'Analyze' > 'Tools' > 'ROI manager', press 'Add', select the ROI, select 'More', select 'Save...'

For movies with multiple frames that have multiple channels and multiple z planes, select 'Image' > 'Color' > 'Split Channels'. Select the channel required for the diameter measurement and convert the movie to a single-plane movie (if it has multiple z planes). To select a single-plane, select 'Image' > 'Duplicate' > tick 'Duplicate hyperstack' > in the 'Slices (z)' box, select the z-plane. Alternatively, movies can be z-projected: select 'Image' > 'Stacks' > 'Z project', select the appropriate projection type and press 'OK'. Select the straight line tool and draw a line over the object for which the diameter measurement is required. Adjust the line width as required. Drag and drop the 'FWHM\_MultipleFrames.ijm' or 'FWQM\_MultipleFrames.ijm' macro into Fiji and press 'Run'. A ‘Warning: Poor Fitting’ message will appear if r2 is <0.6 for the Gaussian fit. The line name, the FWHM or FWQM values (for each frame of the movie) and unit (e.g. μm) will appear in a table. To save the line selection, select 'Analyze' > 'Tools' > 'ROI manager', press 'Add', select the ROI, select 'More', select 'Save...'