3.1.3. SCREEN: Worms are being observed under a higher magnification lens.

Video3\_1: 0:00:03--0:00:09

3.2.1. SCREEN: An initial image is being captured, and signal intensities are being checked using histograms Video3\_1: 0:00:11--0:00:16

3.3.1. SCREEN: The axon is being bleached using a 488nm laser. Video3\_1: 0:02:01--0:02:21

3.3.2. SCREEN: The signal intensity of the axon before and after the bleaching step is being compared.

Video3\_1: 0:02:28--0:02:36

3.3.3. SCREEN: 2 x 2 binning is being performed. Video3\_1: 0:02:47--0:02:52

3.4.1. SCREEN: The exposure time is being increased. Video3\_2: 0:00:03--0:00:17

3.4.2. SCREEN: The temporal resolution is being set to 300-700 ms. Video3\_2: 0:00:12--0:00:17

3.5.1. SCREEN: A time-lapse video is being recorded Video3\_2: 0:00:40--0:01:46

4.2.1. The time-lapse data file is being imported into Fiji. Video4\_1: 0:00:03--0:00:07

4.2.2. The movement or drift of the animal/axon segment is being checked. Video4\_1: 0:00:10--0:00:14

4.2.3. The movement or drift is being corrected using StackReg with rigid body transformation. Video4\_1: 0:00:16--0:01:40

4.3.1. The Segmented Line tool is being used to adjust the line width. The segmented line icon is being selected, and a line is being drawn along the axon segment. Video4\_1: 0:01:41--0:02:04

4.4.1. The ImageJ/Fiji plugin Kymoreslicewide is being used to generate the kymograph with the maximum intensity value across the width of the line. Video4\_1: 0:02:05--0:02:11

4.5.1 The Straight Line tool is being used to trace transport events on the kymograph. Each line is being saved to the ROI manager. TXT: Ensure all transport events in the kymograph are traced Video4\_2: 0:00:06--0:00:21 **TXT: Ensure all transport events in the kymograph are traced** Video4\_3: 0:00:02

4.5.2. SCREEN: Measurement parameters like Area, Bounding Rectangle, Mean Gray Value, Feret's Diameter are being selected. Video4\_3: 0:00:03--0:00:18

4.6.1. The results table is being pasted into a spreadsheet. Video4\_4: 0:00:03--0:00:03

4.6.2. The width is being multiplied by the resolution of the camera. Video4\_4: 0:00:08--0:00:14

4.7.1. The height is being multiplied by the acquisition time between consecutive timepoints. Video4\_4: 0:00:16--0:00:21

4.7.2. The pausing time is being calculated. Video4\_4: 0:00:24--0:00:31

4.8.1. The total events are being normalized to the total length of the kymograph for event number per minute and axon length segment. Video4\_5: 0:00:04--0:00:25

4.8.2. The events are being categorized as anterograde and retrograde transport using the Feret Angle tool. Video4\_5: 0:00:30--0:00:33

4.9.1. The directionality of each event is being determined for kymographs generated by drawing a segmented line from proximal to distal. Video4\_6