Electronic Supplementary Material (ESI) for Lab on a Chip.

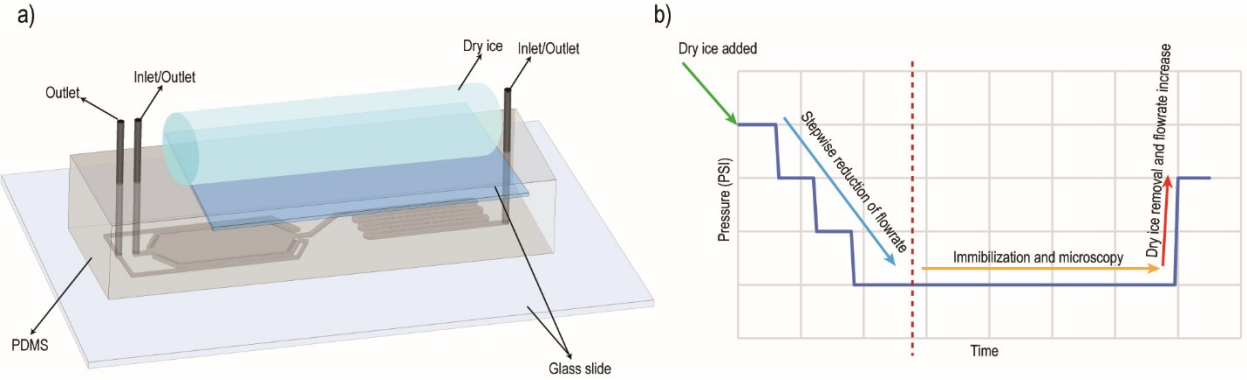

Figure 1 Immobilization schematic. a) The setup for immobilization process where dry ice is placed on the top of the platform. b) The immobilization process flow.

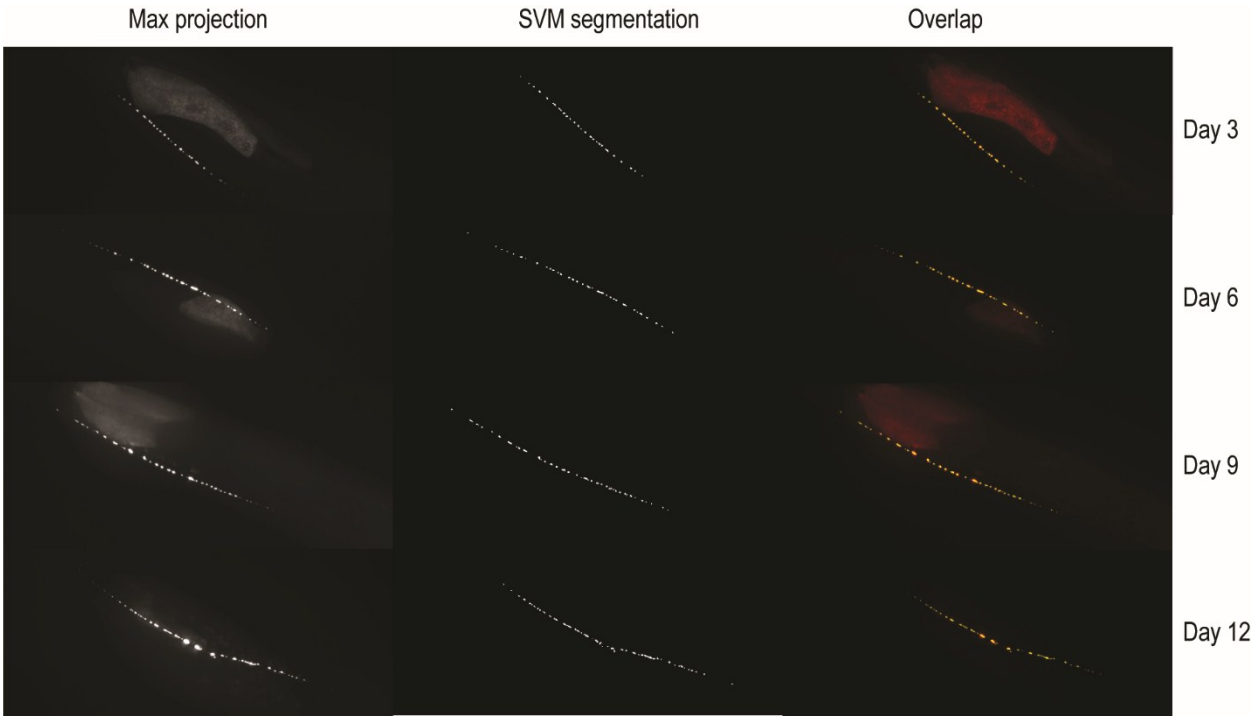

Figure 2 Image segmentation and synaptic detection. Maximum projection images taken for each worm are fed to the segmentation algorithm. SVM segmentation algorithm performs unsupervised image processing by exploiting machine learning techniques. The overlay of the original image and the segmented image isshown in the right coloumn. The red segments are the regions that the algorithm eliminated as background or unwanted parts. The yellow regions are the points that algorithm detected as synapses.

Experimental Setup

Prior to daily progeny evacuation or periodic microscopy, the platform was transferred from the safety cabinet to the inverted microscope. A custom made pressure box provided and regulated the pressurized air required to drive the flow through the device. Before introducing the worms to the device, all channels and chambers were completely degassed by flowing M9 with 0.01% of TX-100 at high-flowrates. The bubbles trapped in the auxiliary channels and imaging channels could impact the progeny evacuation and loading efficiency. Prior to transferring the egg-laying population to the device for filtering the progeny, the clumps formed as a result of eggs attaching to each other were pipetted out to facilitate progeny evacuation by preventing any clogging. To eliminate the probability of carbon dioxide diffusion through the PDMS as dry ice sublimates during microscopy, the top surface of the microfluidic device was completely covered with glass slides. The presence of the coverslips proved that immobilization was temperature induced and not caused by the nematodes' exposure to carbon dioxide which also causes immobilization but has synaptic level side effects[1]. After each run, the containers with worms in them were disconnected from the system and replaced with new conical tubes for the following day's test. The setup was then transferred back to the biosafety cabinet and UV irradiated for 2 hours to eliminate any contamination on the outer surfaces that could be transferred into the process.

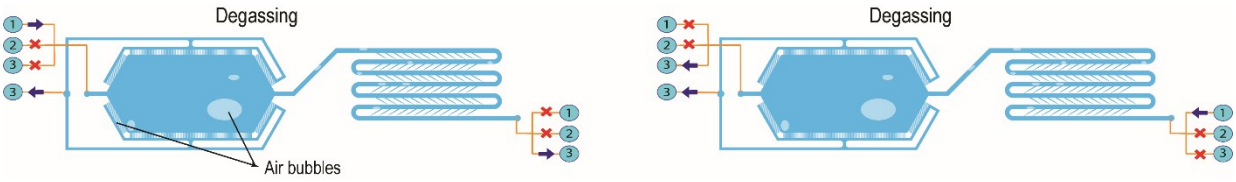

Figure 3 Platform degassing. The device is degassed by flowing pressurized air through it. The high-flowrate will be constantly flown until all air bubbles are evacuated.

List of metrics extracted for quantitative analysis:

1) Number of puncta larger than one pixel.
2) Average size of all the puncta.
3) Average size of the puncta larger than one pixel.
4) $$\frac{\text{Second central moment of the size of the puncta larger than one pixel}}{(\text{Mean of the size of puncta size larger than one pixel})2}$$
5) Number of puncta larger than 8 pixels.
6) Percentage of puncta smaller than 6 pixels.
7) First quartile of puncta size
8) Median of puncta size
9) Third quartile of puncta size
10) 90^{th} percentile of puncta size
11) Maximum puncta size
12) Mean size of the smallest half of the puncta

13) $\frac{\text{Standard deviation of the size of the smallest half of the puncta}}{\text{Mean of the size of the smallest half of the puncta}}$

14) Mean size of the largest half of the puncta

15) $\frac{\text{Standard deviation of the size of the largest half of the puncta}}{\text{Mean of the size of the largest half of the puncta}}$

16) $\frac{\text{90th percentile of the puncta size}}{\text{First quartile of the puncta size}}$

17) $\frac{\text{Mean size of the largest half of the puncta}}{\text{Mean size of the smallest half of the puncta}}$

18) Mean of average puncta intensity (average puncta intensity refers to the mean pixel intensity value for each puncta)

19) $\frac{\text{Standard deviation of average puncta intensity}}{\text{Mean of average puncta intensity}}$

20) Mean of integrated puncta intensity (integrated intensity refers to the sum of all pixel intensity values for each puncta)

21) $\frac{\text{Standard deviation of integrated puncta intensity}}{\text{Mean of integrated puncta intensity}}$

22) $\frac{\text{Second central moment of integrated puncta intensity}}{(\text{Mean of integrated puncta intensity})^2}$

23) Minimum of integrated puncta intensity

24) First quartile of integrated puncta intensity

25) Median quartile of integrated puncta intensity

26) Third quartile of integrated puncta intensity

27) 90^{th} percentile of integrated puncta intensity

28) Maximum of integrated intensity

29) Total distance of synaptic domain (computed by adding distance of individual interpunctal segments larger than 3 pixels)

30) Mean interpunctal distance (ignoring segments smaller than 3 pixels)

31) $\frac{\text{Standard deviation of interpunctal distance (ignoring segments smaller than 3 pixels)}}{\text{Mean of interpunctal distance (ignoring segments smaller than 3 pixels)}}$

32) 90^{th} percentile of interpunctal distance (including all segments)

33) Density, computed by: $\frac{\text{Number of puncta larger than 1 pixel}}{\text{Total distance of synaptic domain (ignoring segments smaller than 3 pixels)}}$

34) Percentage of puncta smaller than 15 pixels and larger or equal than 10 pixels

35) 95th percentile of interpunctal distance (including all segments)

36) Third quartile of interpunctal distance (including all segments)

37) Mean of average interpunctal intensity *(interpunctal intensity refers to the mean pixel intensity value for each interpunctal segment)*

38) $\frac{\text{Standard deviation of interpunctal intensity}}{\text{Mean of interpunctal intensity}}$

39) 10th percentile of puncta size

40) 10th percentile of integrated intensity

41) Fraction of puncta pixels with intensity larger or equal than 500 and smaller than 1000

42) Fraction of puncta pixels with intensity larger or equal than 1000 and smaller than 1500

43) Fraction of puncta pixels with intensity larger or equal than 1500 and smaller than 2000

44) Fraction of puncta pixels with intensity larger or equal than 2000 and smaller than 2500

45) Fraction of puncta pixels with intensity larger or equal than 2500 and smaller than 3000

46) Fraction of puncta pixels with intensity larger or equal than 3000 and smaller than 3500

47) Fraction of puncta pixels with intensity larger or equal than 3500 and smaller than 4000

48) Range of puncta pixel intensity values (computed by subtracting the dimmest pixel value from the brightest pixel value)

49) $\frac{\text{Standard deviation of puncta pixel values}}{\text{Mean of puncta pixel values}}$

50) First quartile of standardized pixel values (standardized pixel values refers to the intensity pixel values where the value of the dimmest pixel has been subtracted)

51) Median of standardized pixel values

52) Third quartile of standardized pixel values

53) 90th percentile of standardized pixel values

54) Fraction of pixels with standardized intensity values smaller than 0.1(Range of pixel values)

55) Fraction of pixels with standardized intensity values smaller than 0.25(Range of pixel values) and larger than 0.1(Range of pixel values)

56) Fraction of pixels with standardized intensity values smaller than 0.5(Range of pixel values) and larger than 0.25(Range of pixel values)

57) Fraction of pixels with standardized intensity values smaller than 0.75(Range of pixel

values) and larger than 0.5(Range of pixel values)

58) Fraction of pixels with standardized intensity values smaller than 0.9(Range of pixel values) and larger than 0.75(Range of pixel values)

59) Fraction of pixels with standardized intensity values smaller than 0.95(Range of pixel values) and larger than 0.9(Range of pixel values)

60) Number of puncta larger than 0.25(Range of puncta size); where range of puncta size is computed by subtracting the smallest puncta size from the largest puncta size

61) Total integrated intensity: sum of all puncta pixel intensity values

Reference

1. Colhoun, E. H. The Physiological Significance of Acetylcholine in Insects and Observations upon other Pharmacologically Active Substances. in (eds. Beament, J. W. L., Treherne, J. E. & Wigglesworth, V. B.) **1,** 1–46 (Academic Press, 1963).