

Automated System Operation

Animals were recorded using a 20×/0.5NA magnification objective in fluorescence mode on a wide field inverted compound microscope (Leica DM IRB/E). Images were captured and analyzed using a digital CCD camera (Hamamatsu ORCA C4742-95-12). All image analysis in this work was programmed using MATLAB®.

The presence of animals within the microfluidic device was detected by computing the average pixel intensity of various regions. These regions were user-defined during each experiment and represented background (β), worm (ω), and correct position (ρ) areas within the camera field of view. These regions were selected after loading a worm into the device imaging area by manually operating the microfluidic chip. An animal was considered to have been successfully detected if the average intensity of the worm region satisfied equation 1 below, where α_1 represents an arbitrary weight based on empirical results.

$$\bar{\omega} \geq \alpha_1 \times \bar{\beta} \quad (1)$$

Similarly, after being detected, an animal was considered to be successfully loaded and ready for imaging if the average intensity of the correct position region satisfied equation 2 below, where α_2 represents an arbitrary weight based on empirical results, and $\bar{\rho}_{\text{worm}}$ represents the average intensity of the correct position when a worm was correctly loaded. Animals detected in the correct position region have either their head or tail in close proximity to the end of the imaging area, indicating that the nematode is fully loaded into the curved area of the device.

$$\bar{\rho} \geq \alpha_2 \times \bar{\rho}_{\text{worm}} \quad (2)$$

Loading an animal was performed by sending a command to off-chip solenoids to actuate the on-chip valve configuration and input flow required for positioning. Once an animal was detected in the imaging area of the device by software, it was given a period of four seconds to reach the correct region for image capture and analysis. The system began image acquisition if the nematode was detected in the correct position or if the allotted time had expired.

After image capture, animal phenotype was analyzed to determine the number of D-type motor neurons present using a four step process: (1) images were smoothed using an anisotropic diffusion filter; (2) the smoothed image was segmented for features of interest using global thresholds; (3) potential neurons were filtered by shape and size; and (4) a mean and size filter was utilized to remove objects falsely detected as neurons.

For this study, an animal was considered to be a suppressor of the *smn-1* neurodegenerative phenotype if 10 or more D-type motor neurons were detected within a specified analysis area.

Once image analysis and phenotype classification was complete, the nematode was sorted to the appropriate mutant or wild-type output using off-chip solenoids to actuate the appropriate on-chip valve

configuration and activate fluid flow in both the input and flush channel. Once an animal was no longer detected, the entire process of loading, phenotype analysis, and sorting was repeated.

All time limits for system processes were set to values based on empirical data. All animal detection and phenotype analysis was performed with the microscope in fluorescence mode. System operation and software decisions were executed by polling the status of worm detection software modules at a rate of 30Hz.

Table 1: Isolated Alleles Summary

Isolated Allele	Number of Motor Neurons		
	Mean	Std	% ≥ 15
Non mutagenized (n=60)	9.3	2.8	6.7%
<i>a182</i> (n=25)	12.6	2.3	26.1%
<i>a183</i> (n=24)	13.4	2.0	32.0%
<i>a184</i> (n=23)	12.9	2.5	26.1%
<i>a185</i> (n=21)	11.8	3.9	33.3%
<i>a186</i> (n=25)	14.2	2.0	56.0%
<i>a187</i> (n=25)	16.5	1.2	92.0%
<i>a188</i> (n=25)	15.1	2.6	64.0%
<i>a189</i> (n=25)	15.3	2.4	68.0%
<i>a190</i> (n=25)	17.0	1.4	92.0%
<i>a192</i> (n=25)	15.2	2.2	76.0%
<i>a193</i> (n=25)	14.9	1.6	64.0%
<i>a194</i> (n=25)	14.5	2.0	60.0%
<i>a195</i> (n=25)	17.5	1.2	96.0%
<i>a196</i> (n=25)	15.2	1.3	60.0%
<i>a197</i> (n=25)	12.3	2.0	12.0%
<i>a198</i> (n=25)	14.5	1.9	56.0%
<i>a199</i> (n=25)	16.0	1.8	76.0%
<i>a200</i> (n=25)	12.4	2.4	16.0%
<i>a201</i> (n=25)	16.7	1.1	100.0%
<i>a202</i> (n=25)	12.6	2.4	24.0%
<i>a203</i> (n=25)	16.2	1.7	80.0%