Developing microfluidic platforms for rapid identification of new targets for neurodegenerative disorders

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Neuromuscular disorders encompass a range of diseases that affect the peripheral nervous system. *Caenorhabditis elegans* (*C. elegans*) is an attractive source for whole-animal phenotypic screens as its size, transparency and complete nervous system allow for clear visualization of all cells during in-vivo experimentation [1]. Conventional drug screening methods are slow and laborious, and *C. elegans* neurodegenerative disease models are used for drug screening to elucidate the putative cellular/molecular networks that are disturbed in diseased models. By combining microfluidics, commercial imaging platforms and data analysis software, *C. elegans* can be amenable to high-throughput screening approaches, allowing rapid testing of potential treatment options for various neuromuscular disorders, including the motor neuron disorder spinal muscular atrophy (SMA) [2].

This paper presents a microfluidic, PDMS platform, manufactured using rapid prototyping methods for high-throughput visualization and analysis of *C. elegans* (Figure 1). 3D printing techniques are used to rapidly manufacture molds with typical timeframes from conceptualisation to realisation in less than twelve hours. The transparency and robustness of PDMS [3] permits the visualisation of *C. elegans* via standard inverted microscopy techniques and is compatible with commercial software tracking systems. This is of particular importance since *C. elegans* contingent movement can give rise to artifacts or out of focus images when using conventional microscopy making observations difficult. PDMS platforms are ideal for morphological and phenotypic investigation as they allow for ease of animal handling and manipulation and are capable of mechanically trapping numbers of *C. elegans* at a once [4]. Current drug screening assays are based on image-based observations with the focus on morphological-descriptive traits like locomotory parameters such as swimming (thrashing), which is a critical biophysical parameter used to assess neuromuscular function in *C. elegans* when modelling neuromuscular-related diseases. This movement is heavily compromised in *C. elegans* neurodegenerative models and is easily quantifiable using automated tracking systems that can determine changes in position and shape, frame by frame (e.g,WormLab®, MBF Bioscience).

The swimming (thrashing) behavior of *C. elegans* has traditionally been performed by placing animals in a drop of PBS and manually counting the number of body bends (Figure 2A). This method is laborious and subject to the researcher's ability to identify and count, in situ, the number of waves propagating in *C. elegans* body. Five devices were manufactured using different parameters for software compatibility and the density of animals that could be simultaneously imaged were tested (Figure 2B1, 2B2 & Figure 3). Shadow correction was applied during filming as shadows, due to the imperfections from manufacturing, were interfering with the analysis (Figure 2B3). Recordings loaded into the WormLab® software (Figure 4B) with data corresponding to swimming behavior were then exported into a preferred format for posterior statistical analysis (Figure 4C).

Device 5 was used in the final recordings due to the (a) density of worms analysed in a single frame at the required magnification and the (b) reduction of shadowing observed. The new platform can track 20 animals simultaneously using WormLab® software allowing for multiple screenings of neurodegenerative diseased *C. elegans* models.

Word Count: 498

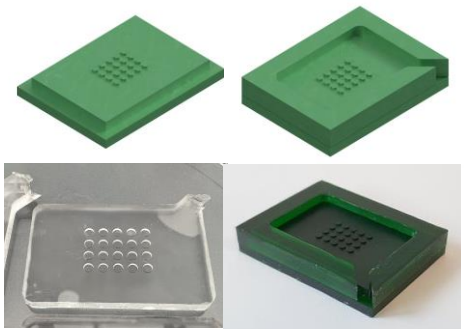

Figure 1*: CAD image of microchambers molds for C. elegans inspection. 3D printed mold and PDMS replica. Mold was printed using CADworks 3D microfluidics printer in master mold resin.*

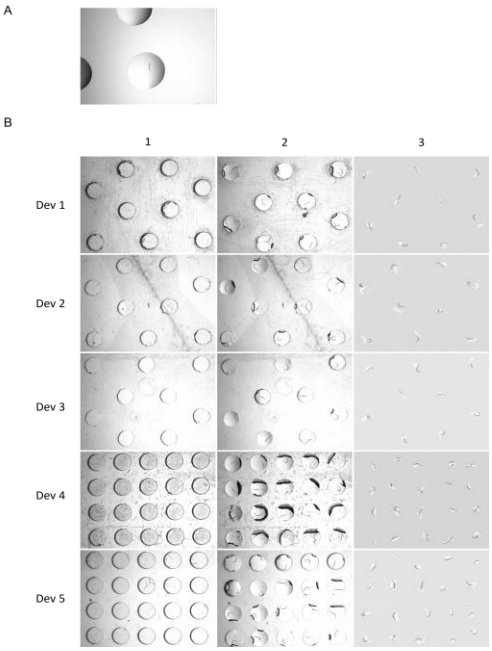

Figure 2*. Devices tested to assess C. elegans swimming (thrashing) behaviour. (A) Single animal in PBS (5 µL). (B1) Empty chambers. (B2) Chambers loaded with 2 µL PBS and single animals placed in the corresponding wells. (B3) Final images captured with a 63x objective using AxioCam ICc5 camera on a Discovery V8 SteREO microscope.*

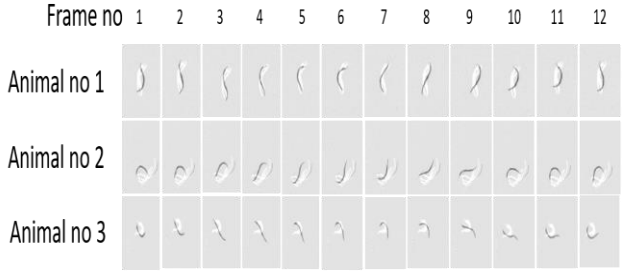

Figure 3. *C.elegans swimming behaviour. Animals in PBS were filmed at 175 frames for 10sec over 12 consecutive frames.*

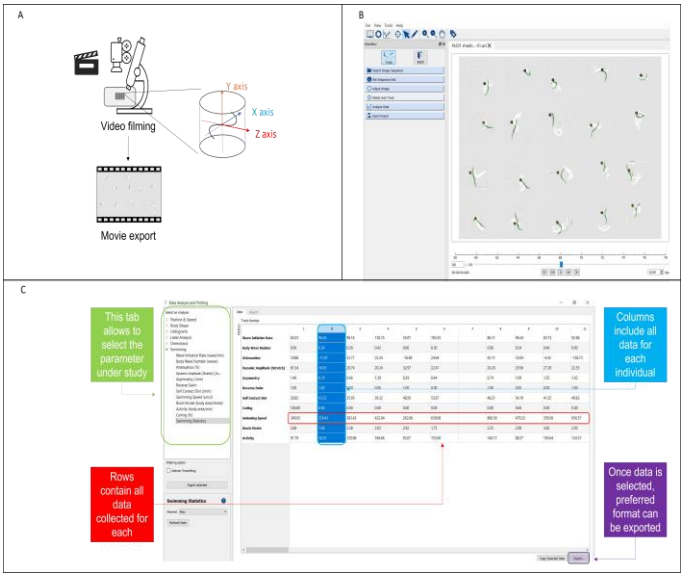

Figure 4*: Experimental design for C. elegans swimming (thrashing) assay. (A) Microscope and platform setup. Animals lay on the X axis and thrash in the Z axis for the posterior analysis. Ten second videos are captured and exported as movies into WormLab® (B). (C) Various parameters analysed.*

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