QUANTITATIVE PHENOTYPING OF C.ELEGANS BEHAVIOR IN AN AUTOMATED MICROSYSTEM

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ABSTRACT

Caenorhabditis elegans is an excellent model system for studying the genetics and neuronal basis of behavior. Studying *C. elegans* behavior gives insight into how networks of neurons integrate environmental cues to produce specific behavior. Current behavior experiments are labor intensive and lacking precise control of the stimuli. This manual behavior analysis can introduce bias, is less quantitative and low in throughput due to limited statistics. Here we present an automated microsystem for quantitative population experiments. Worms are placed in a pillar gradient device made of polydimethylsiloxane (PDMS) to study the effect of ethanol on locomotion and chemotaxis.

KEYWORDS: *C. elegans*, Microfluidics, Behavior, Phenotyping

INTRODUCTION

Caenorhabditis elegans, a soil nematode, is an excellent model system for studying the genetics and neuronal basis of behavior [1] due to its transparency, the availability of many molecular and genetic tools, and strong homologies to higher organisms [2]. Because of the small number of neurons (302 in hermaphrodite) and rich behavior repertoire, studying *C. elegans* behavior gives insight into how networks of neurons integrate environmental cues to produce specific behavior. Current behavior experiments are labor intensive (often requiring single-worm tracking and manual scoring of behavior) and lacking precise control of the stimuli [3]. Manual behavior analyses can introduce bias, are less quantitative and low in throughput due to limited statistics. Here we present an automated microsystem for quantitative population experiments to study the effect of small molecules on behavior.

THEORY

C. elegans crawl in a periodic S-shape; the device was designed to allow worms to mimic crawling conditions in solution. To increase the throughput of experiments and exaggerate behavior or interest, a step gradient of increasing pillar density was designed into the main chamber (see Table 1). This setup is ideal to study population behavior under chemical influence but can also track individual worms.

Table 1. Distance between pillars for each area (all pillars are 125µm in diameter)

Pillar Area	1	2	3	4	5
Pillar Spacing (µm)	300	245	180	125	80

The device is a one-layer PDMS chip, molded from a SU-8 master, and then bonded to a glass slide (Fig. 1). Because of the large size of the device, a custom setup was created to raster scan the device. Worms are loaded into the device and

tracked by an automated system at 0.1-15 Hz depending on the experiments. Included in this system is a zoom lens and camera attached to a custom built motorized XY stage adopted from G. Stephens *et al.* [4].

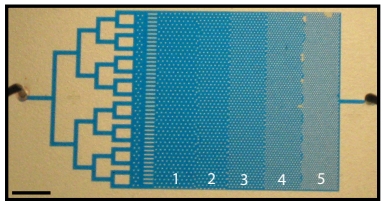
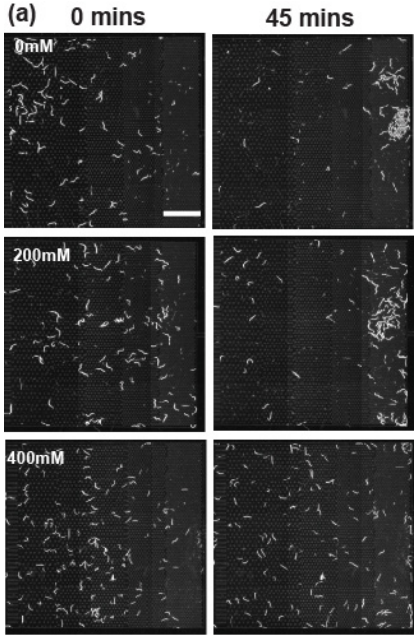


Figure 1. Image of device which includes branched channel loading area and pillar area labeled 1-5 for increasing density (scale bar is 3.5mm).



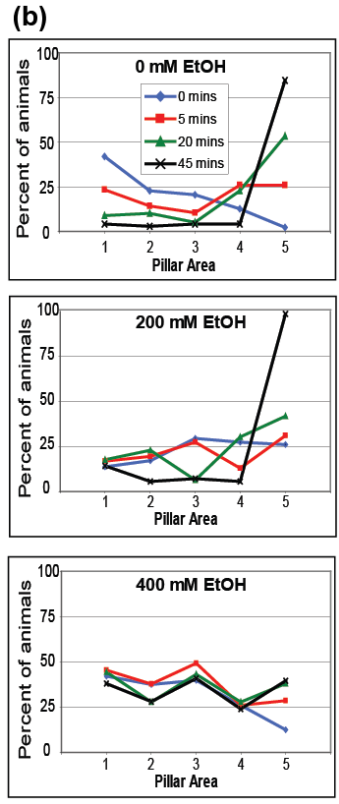


Figure 2. (a) Representative images demonstrating worm migration under the influence of different ethanol concentrations. The scale bar is 3.5mm. (b) Time evolution of the spatial distribution of worms under the specified conditions.

The system is automated using a LabVIEW program to capture images based on magnification, length of experiment, and preset interval, and produce stitched images of the entire device. The advantages are that little post-processing is necessary and multiple worms can be tracked and counted allowing for quantitative standardized population assays over time.

EXPERIMENTAL

To demonstrate the capabilities of this system, ethanol's effect on *C. elegans* behavior was examined. Ethanol is known to affect locomotion (e.g. crawling speed) and body shape (e.g. periodicity of the S shape) [5]. Wild-type animals (N2) under the influence of 0mM, 200mM, and 400mM were tracked for 45 minutes at 5-min intervals (Fig. 2a).

RESULTS AND DISCUSSION

The N2 worms at 0mM ethanol showed a dramatic and consistent attraction toward the densest pillar area (Area 5), which we attribute to aerotaxis towards a low concentration of oxygen that they prefer [6]. In contrast, at the 400mM condition the worms showed no signs of this migratory behavior. This may be due to their hindered locomotion or possible interference of oxygen sensing by ethanol. In our setup, it is easy to track the time evolution of the population distribution (Fig 2b). In addition, velocities of individual worms can also be obtained. Figure 3 shows that pillar density has little effect on animal locomotion at high ethanol concentration.

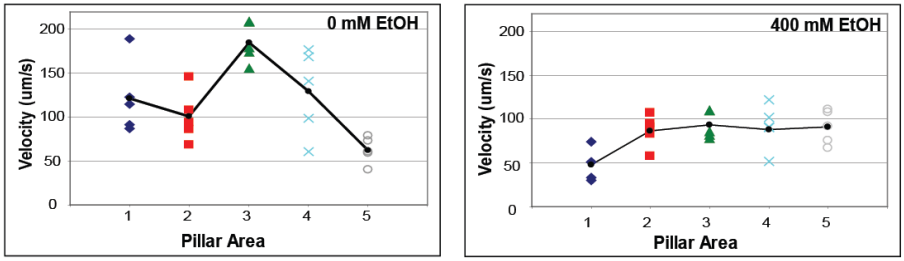


Figure 3. Average velocity of 5 individual worms in each pillar area.

CONCLUSIONS

We envision this system, with further integration of functions, to extract population information in a high throughput manner for behavioral screens of chemicals and small molecules.

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