A Microfluidic Diode for Sorting *C. elegans*

Tao Hong[a], Lijie Yang[b], Richard Mu[c], Guillermo Sanchez[d] and Deyu Li[b]

Queensborough Community College[a], City University of New York, Bayside, NY 11364
Department of Mechanical Engineering[b], Vanderbilt University, Nashville, TN 37235
Life and Physical Sciences Department[c], Fisk University, TN 37208
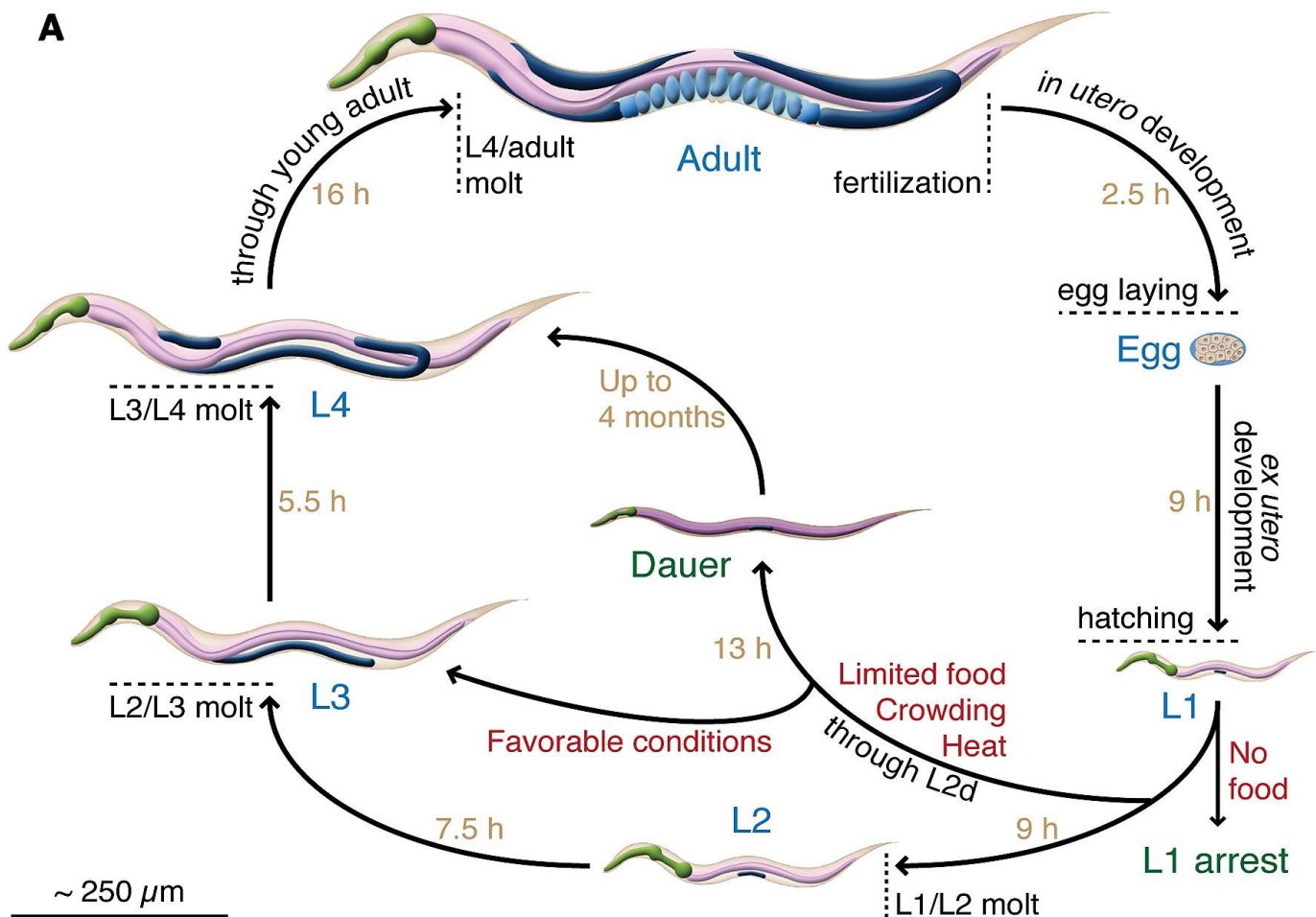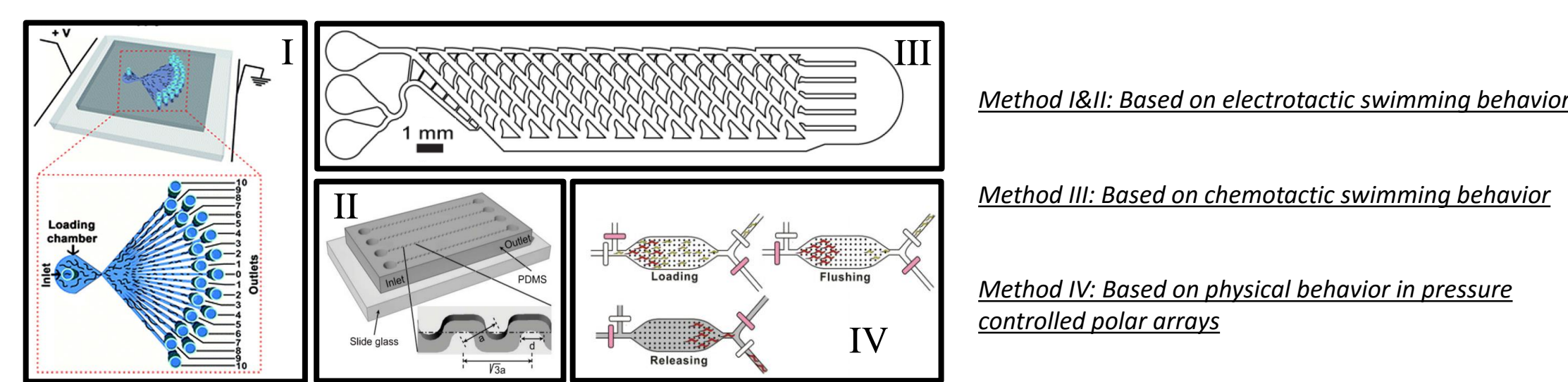Hughes-Kellogg Biology Research Laboratory[d], Fisk University, TN 37208

Introduction

- *Purpose of sorting C. elegans in different developmental stages*

❖ *Caenorhabditis elegans* (*C. elegans*) has been widely used as a powerful model organism which develops from hatching to reproductive adulthood through four larval stages (L1 to L4) and exhibits distinctive stage-specific features.

- *State of art sorting strategy of C. elegans by microfluidic platforms*

Method I&II: Based on electrotactic swimming behavior
Method III: Based on chemotactic swimming behavior
Method IV: Based on physical behavior in pressure controlled polar arrays

Limitation: 1. Narrow sorting region (Except in method I, those devices can separate only two parts of worms, adults and the other larva stages)
2. Additive environmental stimuli (Except in method IV, attractants and electric field are applied in the device which will increase the complexity of operation)

- *Our sorting strategy: Based on crawling behavior in different sized microfluidic diodes*

	L1	L2	L3	L4	Adult
Average diameter	11.7±0.2 μm	17.0±0.2 μm	22.2±0.3 μm	29.6±0.6 μm	47.9±0.8 μm
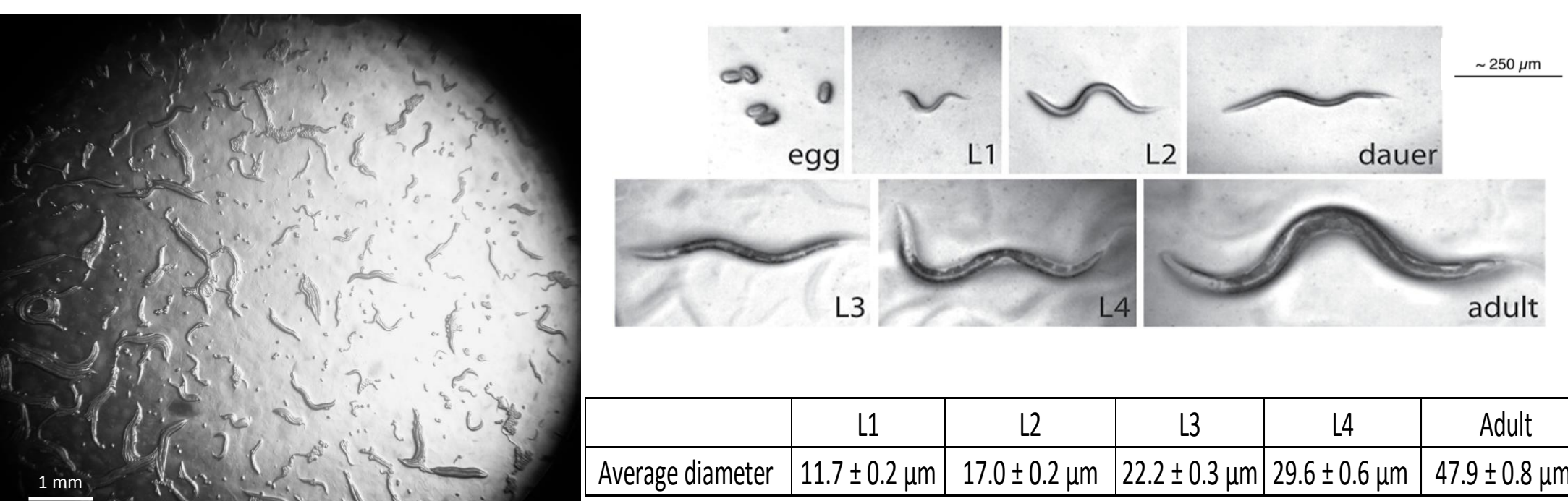
Advantage: 1. Significantly higher accuracy of sorting in L4 to adult region (capable of dividing young adults into four different sections with ~5 μm diameter difference)
2. Different groups of similar sized C. elegans can be separated and trapped into different sections, this property can be used to study the responsive difference to the chemicals in different stages.
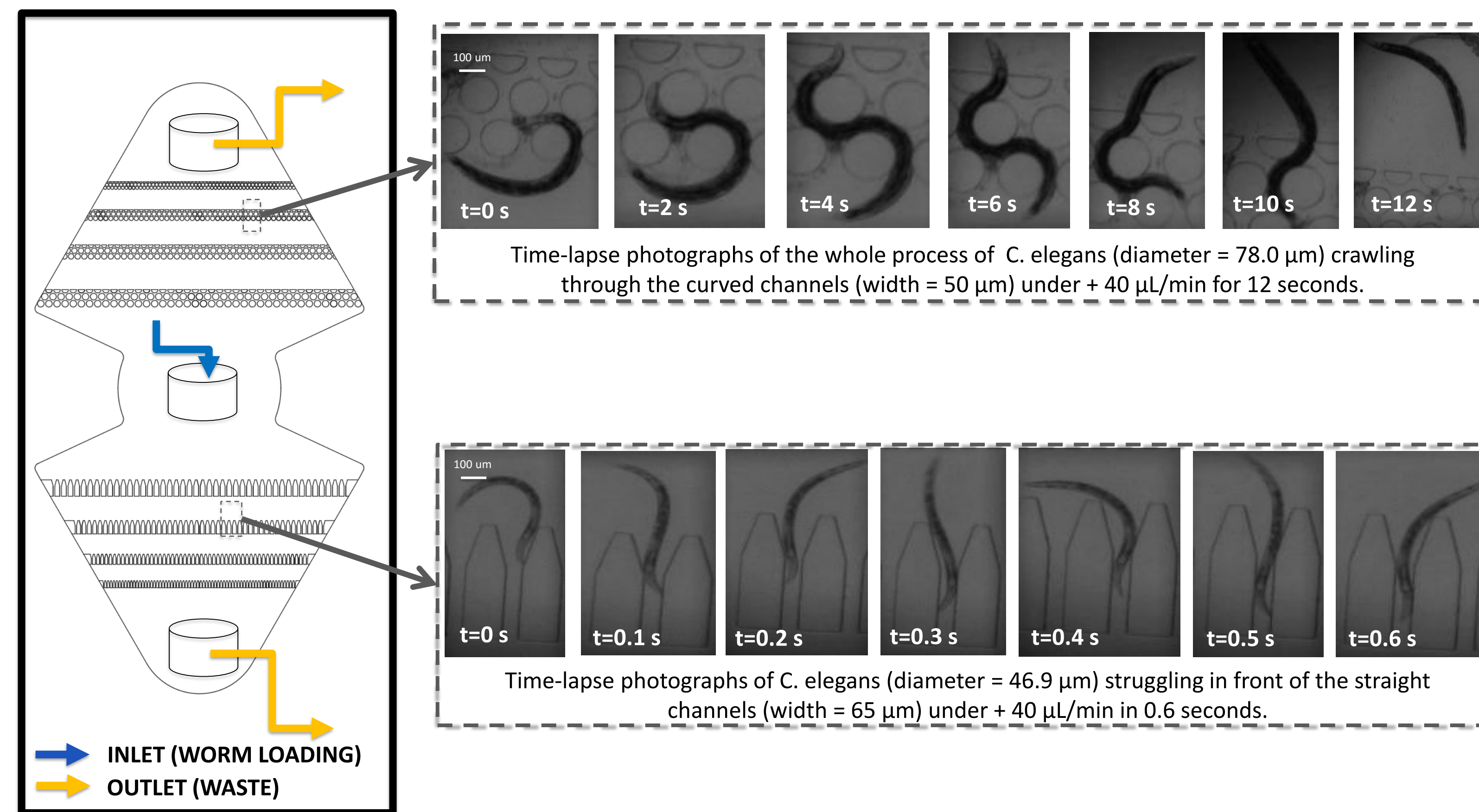
Methods

- *Fabrication of microfluidic devices*

Photolithography

Front view of experiment set up under the microscope
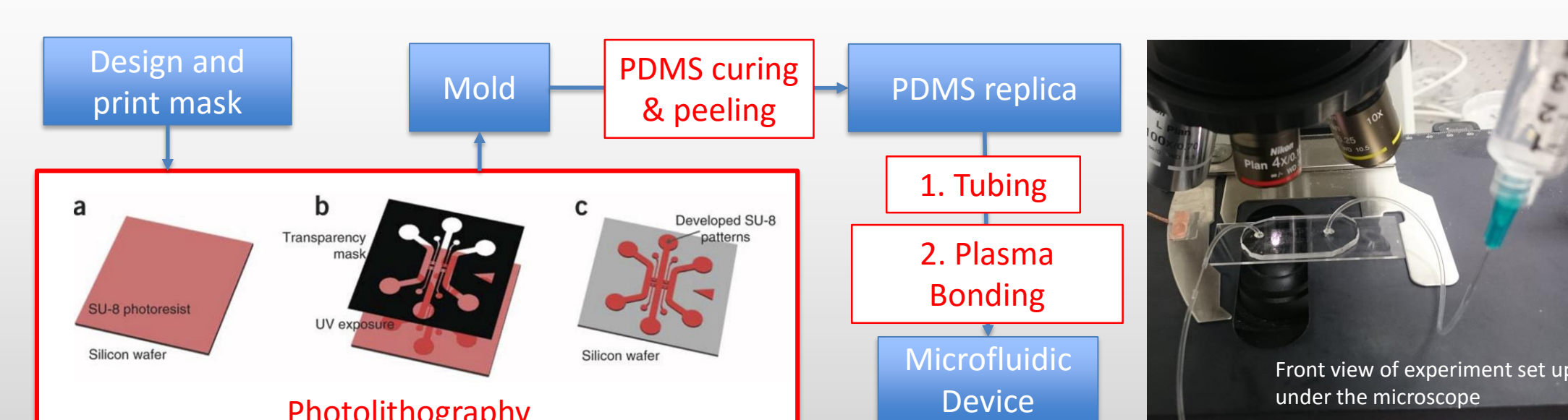
- *Source of unsynchronized C. elegans colony*

The initial unsynchronized C. elegans colony (N2, Wild Type) was provided by Hughes-Kellogg Biology Research Laboratory, Fisk University. The sample colony used in the sorting experiment was cultured from initial colony for 48 hours in 21℃ to reach the maximum population.
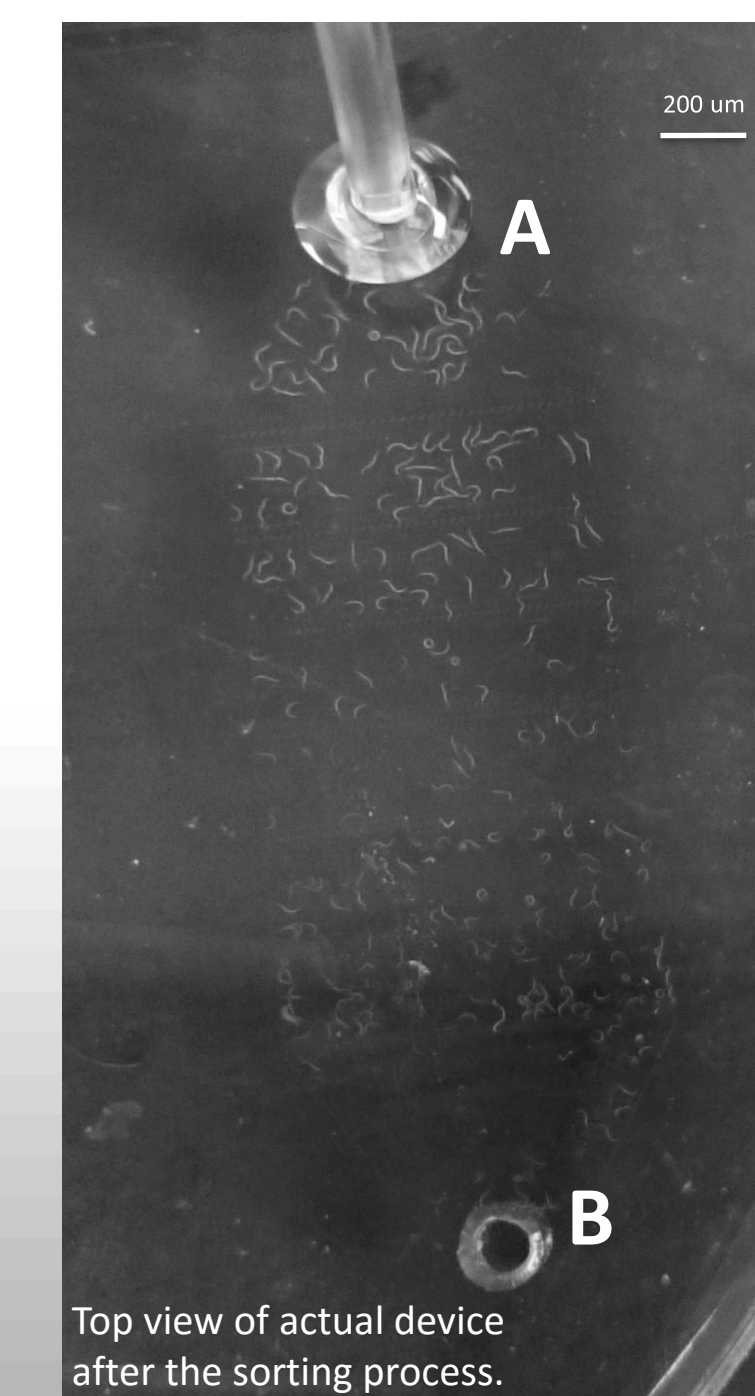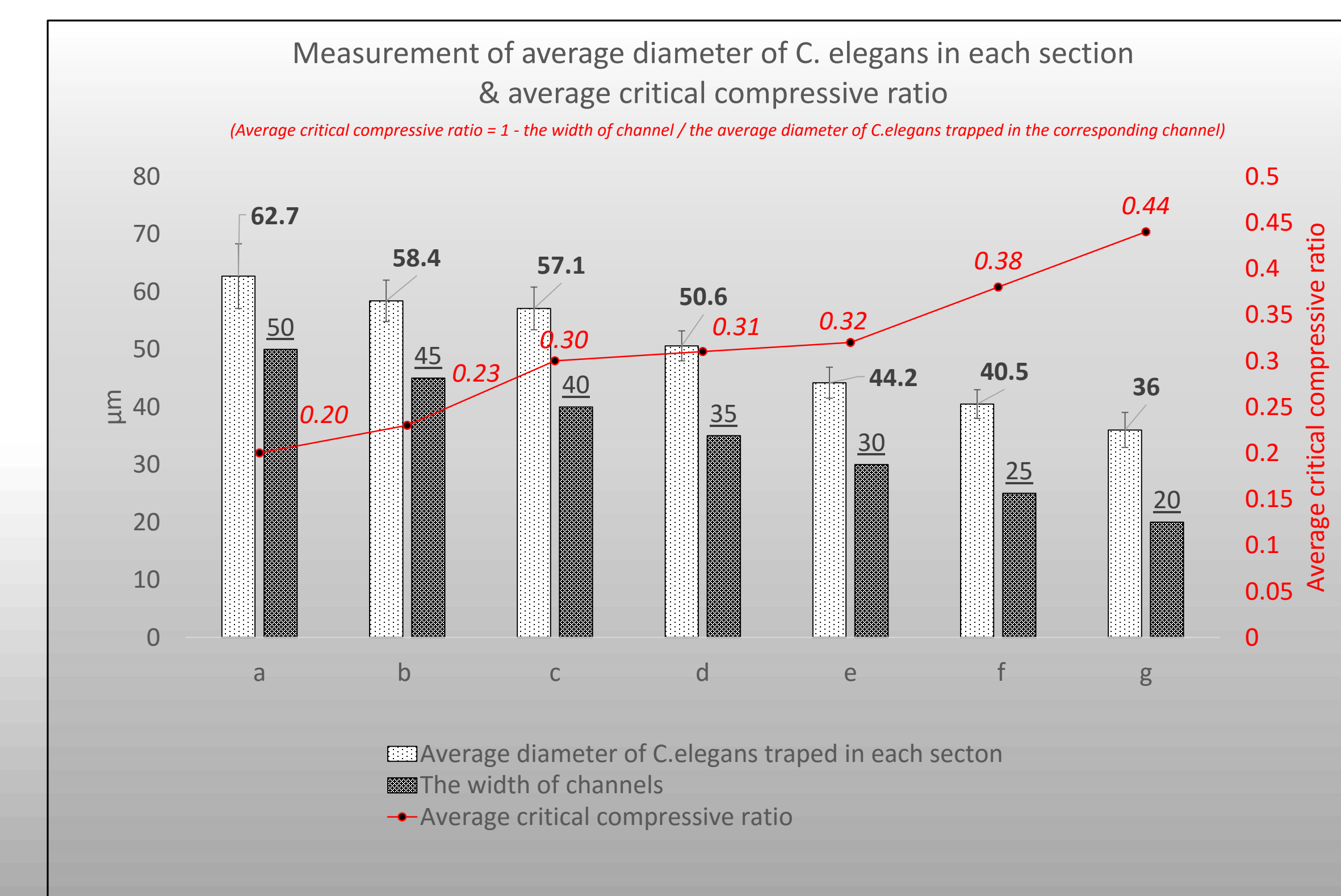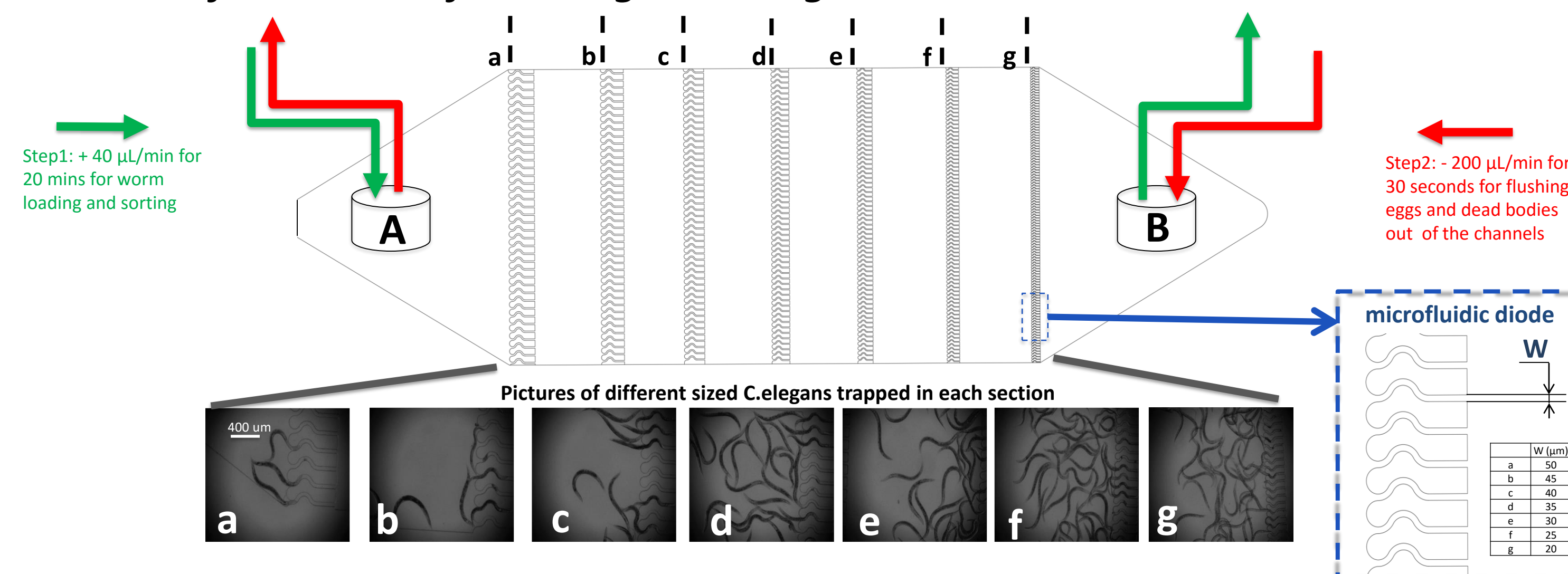
The petri dish cultured C. elegans
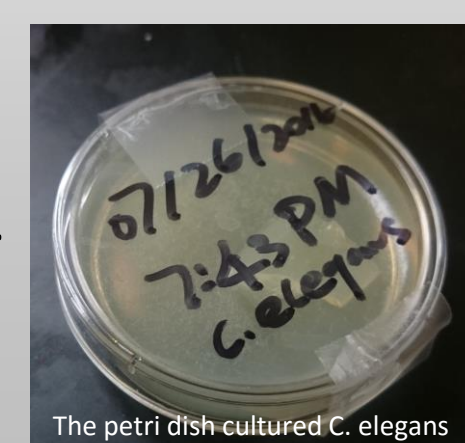
Design and Result

- *Different crawling performance of C. elegans in curved and straight channels*

Time-lapse photographs of the whole process of C. elegans (diameter = 78.0 μm) crawling through the curved channels (width = 50 μm) under + 40 μL/min for 12 seconds.

Time-lapse photographs of C. elegans (diameter = 46.9 μm) struggling in front of the straight channels (width = 65 μm) under + 40 μL/min in 0.6 seconds.

INLET (WORM LOADING)
OUTLET (WASTE)

- *Microfluidic diode for C. elegan sorting*

Step1: + 40 μL/min for 20 mins for worm loading and sorting

Step2: - 200 μL/min for 30 seconds for flushing eggs and dead bodies out of the channels

microfluidic diode

Pictures of different sized C.elegans trapped in each section

Measurement of average diameter of C. elegans in each section & average critical compressive ratio

(Average critical compressive ratio = 1 – the width of channel / the average diameter of C.elegans trapped in the corresponding channel)

- Average diameter of C.elegans traped in each secton
- The width of channels
- Average critical compressive ratio

Top view of actual device after the sorting process.

The impact of different flow rate on the average critical compressive rate in each section
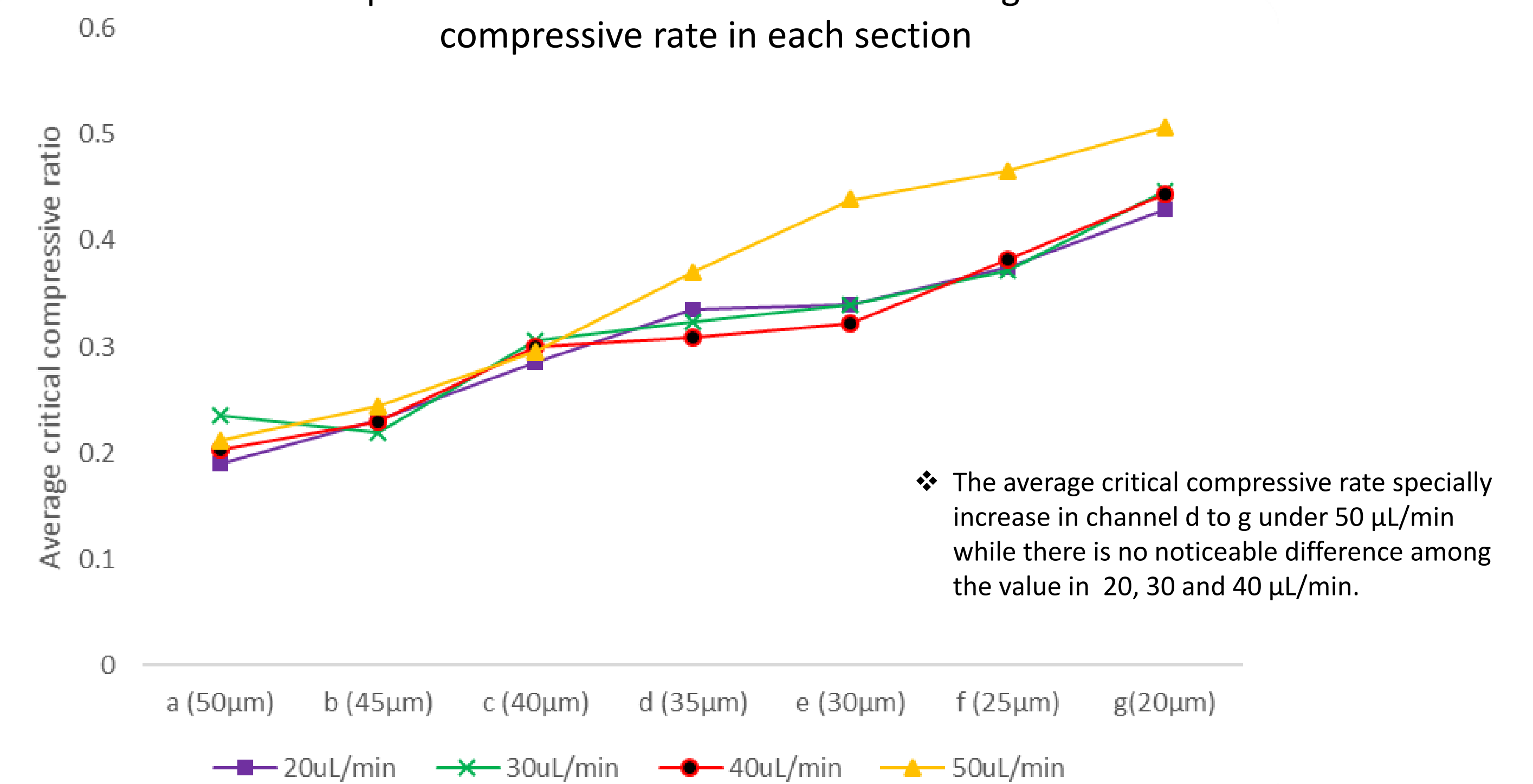
❖ The average critical compressive rate specially increase in channel d to g under 50 μL/min while there is no noticeable difference among the value in 20, 30 and 40 μL/min.

- 20uL/min — 30uL/min — 40uL/min — 50uL/min

Conclusion

❖ C. elegans (L4 and adult) have a spontaneity to compress themselves 20%-50% to penetrate the curved channels, but they cannot penetrate the straight channels with the same width under the same flow rate.

❖ After sorting process, the C. elegans from L4 and Adult (diameter: 31-72 um) are divided in 7 sections based on their diameters. The difference in average diameter of C. elegans in each section is about 5 um.

❖ The average critical compressive ratio increases while the width of the channel decreases, which demonstrates that larva C. elegans can compress more than adult C. elegans in the unidirectional channels.

❖ Increasing flow rate might result in a significant enhancement of average critical compressive ratio in some specific channels.

Future Work

❖ Widen the region of sorting by fabricating channel arrays in smaller width.

❖ Design a collecting system for repeatable use of the device.

❖ Study the impact of curved channels' shape and the length of the straight channels on the diode's property.

❖ Introduce chemical stimuli to the C. elegans trapped in different sections of the original device and study their difference of response.

Acknowledgements

References

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