Go to the worm; consider its ways and be wise![+]

Haim H. Bau

bau@seas.upenn.edu

Mechanical Engineering & Applied Mechanics

University of Pennsylvania

[+] My talk's title rephrases the Bible, Proverbs 6:6 (dated between 1200 and 165 BC)

"לֵךְ-אֶל-נְמָלָה עָצֵל; רְאֵה דְרָכֶיהָ וַחֲכָם."

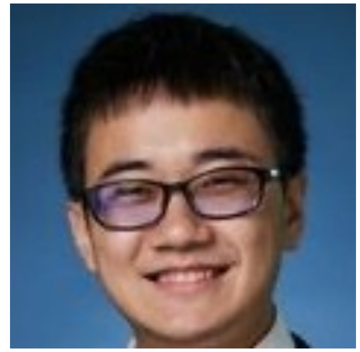
08:55

Acknowledgments

Han-Sheng (Oswald) Chuang, PhD Former postdoc. Currently: Professor, Biomedical Engineering, National Cheng Kung University (NCKU)

Jinzhou Yuan, PhD Former PhD student. Currently: Principal Scientist · Instil Bio

Hungtang Ko. Former MS student. Currently: Postdoc at Princeton Univ.



Wei-Long Chen. Visiting doctoral student. Currently: Biomedical Engineering, NCKU

The main hero C. elegans

Prof. David Raizen, Dept. Neurology, School of Medicine, UPenn

Funding: NIH, UPenn

Worms: *The Good, the Bad and the Ugly*

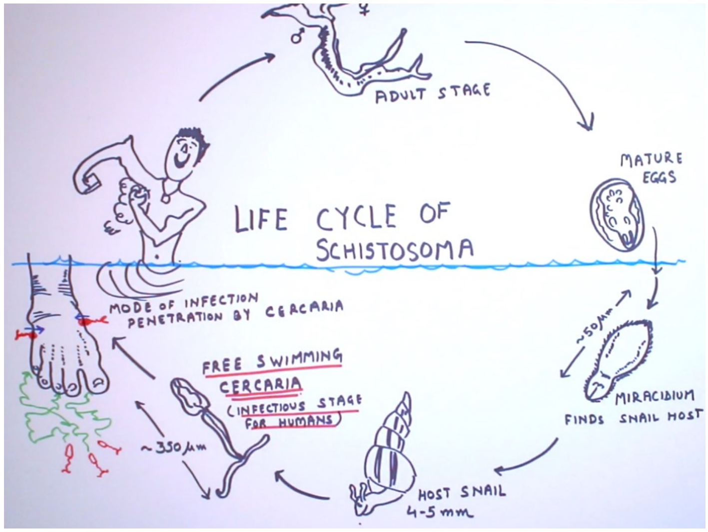
Worms are ubiquitous; over *a **third*** of animal species.

Earth worms modify soil properties for the better and for the worse.

Parasitic worms infect over a **billion humans** and countless livestock and plants, causing morbidity and severe economic damage, estimated at $100 billion annualy in the USA alone.
"… God provided a worm, which chewed the plant so that it withered" (Jonah 4, 7).

The **free-living** (*non-parasitic*) nematode (round worm) *Caenorhabditis* (***C***) *elegans* is a **model animal** used in medical research to identify the **genetic mechanisms** governing animal physiology, development, the aging process and for drug discovery. *C. elegans* and humans share many **homologous genes.**

C. elegans contains about **20,500 genes** like humans



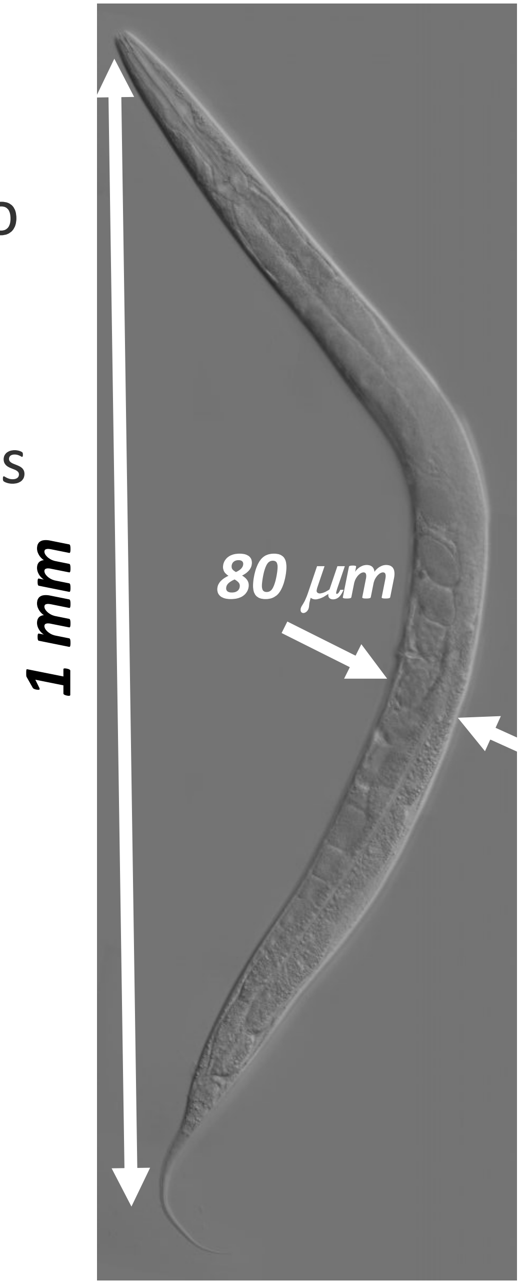
http://gfm.aps.org/meetings/dfd-2014/5416413369702d585c3f0100

Plant parasitic nematode on corn root.
http://www.ipm.iastate.edu/ipm/icm/node/611

Hookworm inside the host.
http://en.wikipedia.org/wiki/Hookworm

Why *C. elegans*?

- Well-mapped nervous system; 302 of *C. elegans*' 959 cells are neurons
- Genome completely sequenced and 40% similar to humans.
- Ability to switch neurons on / off (**optogenetics**)
- Many physiological properties and molecular paths are like in higher animals and humans.
- Ability to replicate human diseases (immune system, Parkinson, …….)
- Transparency, individual cells can be imaged.
- Availability of a large library of mutants (deficient in various genes)
- Amenability to high throughput screens
- Ease of cultivation and short life span (~2 weeks).

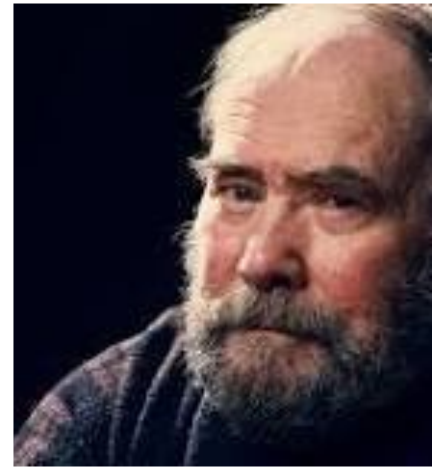


08:55

Nobel Prizes

Many important medical discoveries were made through research with *C. elegans*, including gene editing *(**6 Nobel prizes**)*.

Sydney Brenner. Nobel Prize in Physiology in 2002 for his worm research and for identifying key genes regulating organ development and programmed cell death … and [it] shed new light on the pathogenesis of many diseases.



Sydney Brenner
1927- 2019

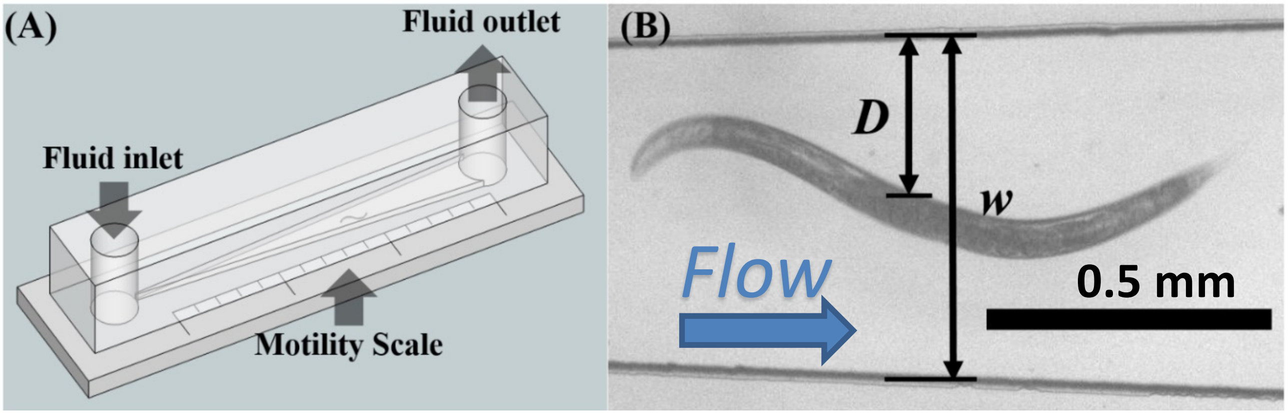
The Nematode Dynamometer

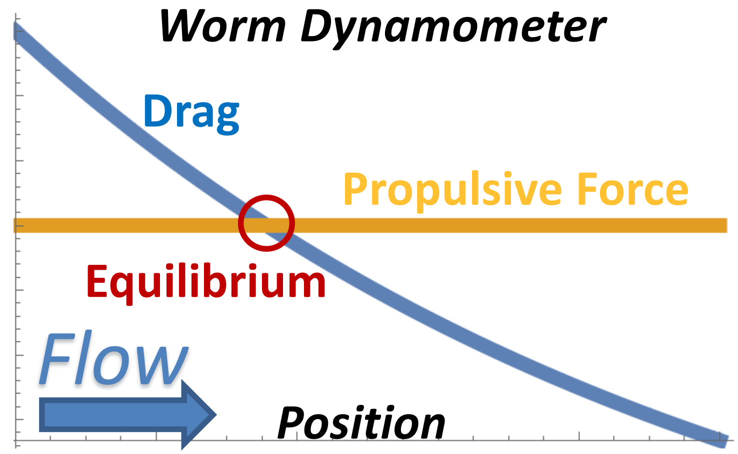
Power and thrust are indicators of health. Power measurements are used in engine testing to test the engine's health.

Likewise, nematode power and thrust are indicators of state of health; susceptibility to toxins; and means to determine drug efficacy.

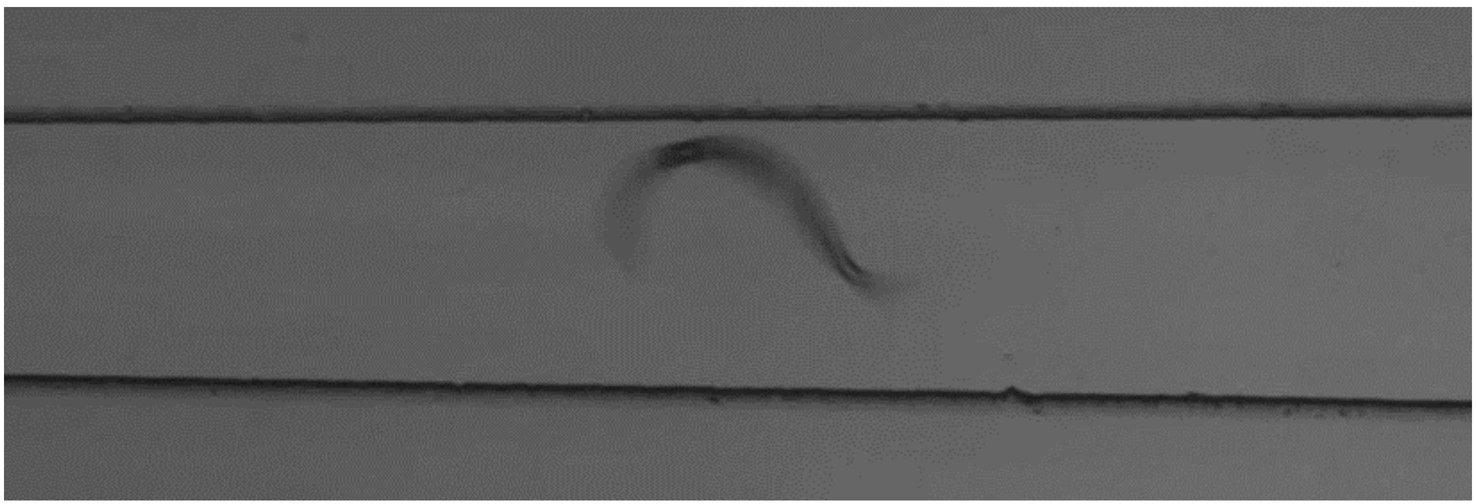
Challenge: how to measure the worm's thrust?

Motility Assay: INFINITY POOL APPARATUS





Worm equilibrium position indicates its propulsive power

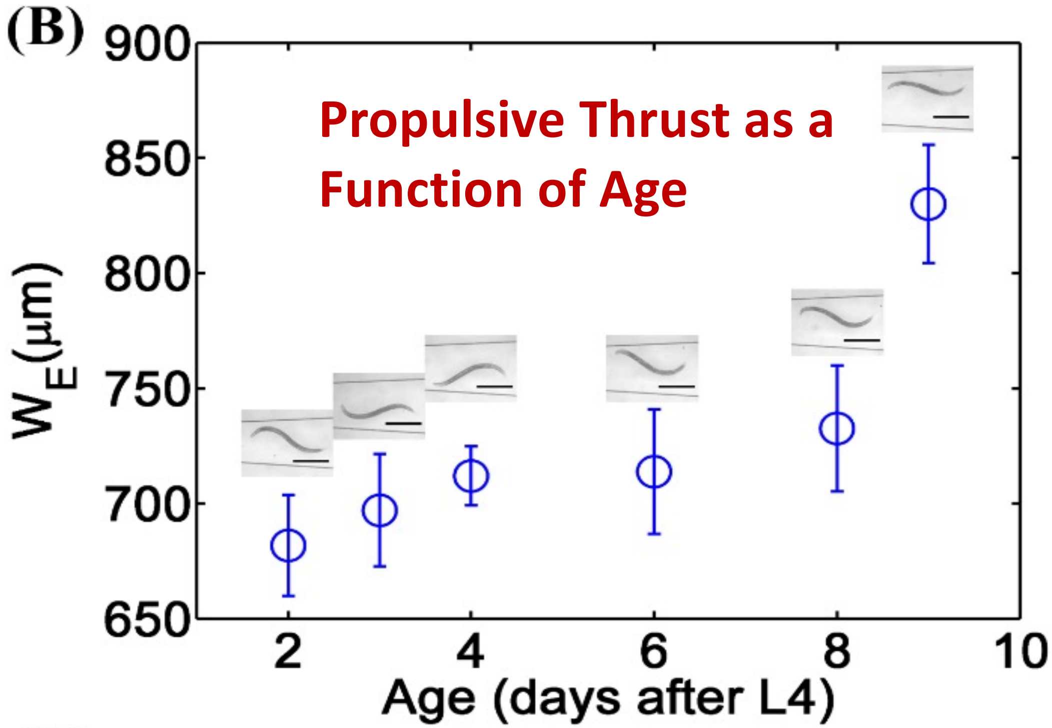


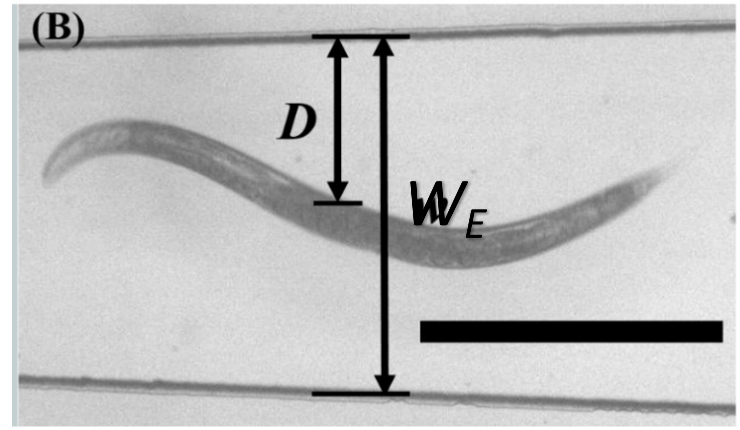
Infinity pool is used to train Olympian swimmers

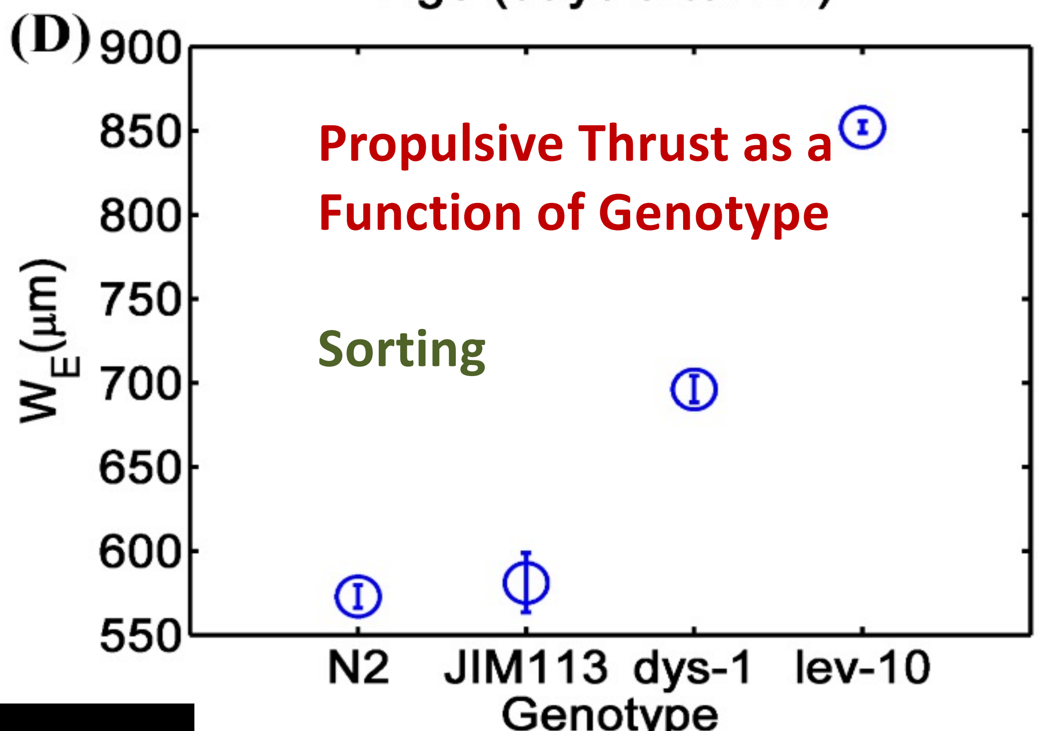
Motility depends on the animal's health and susceptibility to drugs (drug / toxicity screening); efficacy of therapies, and aging

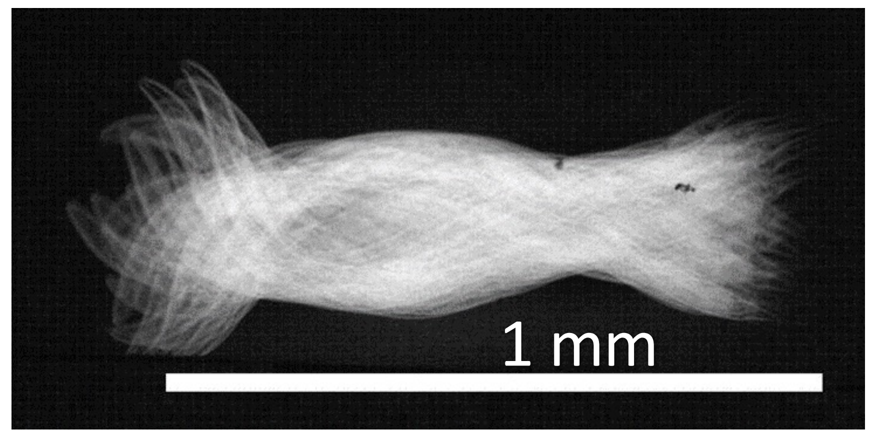
Exercise machine: examine the effect of exercise on longevity

Nonintrusive Localization of animals for observation and intervention









The infinity pool apparatus localizes the animal for prolonged observations without impeding its motion

08:55

Research inspired by teaching

Why do many students fall asleep in class while a few stay awake?

Hypothesis: sleeplessness (insomnia) is caused by a genetic disorder. If we discover the gene(s) associated with the lack of sleep, perhaps we can design a therapy.

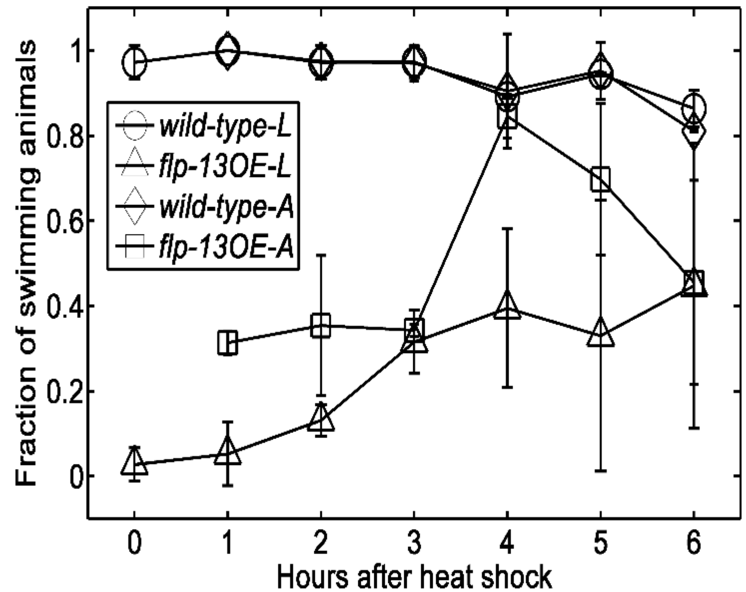
08:55

Forward Genetic Screen

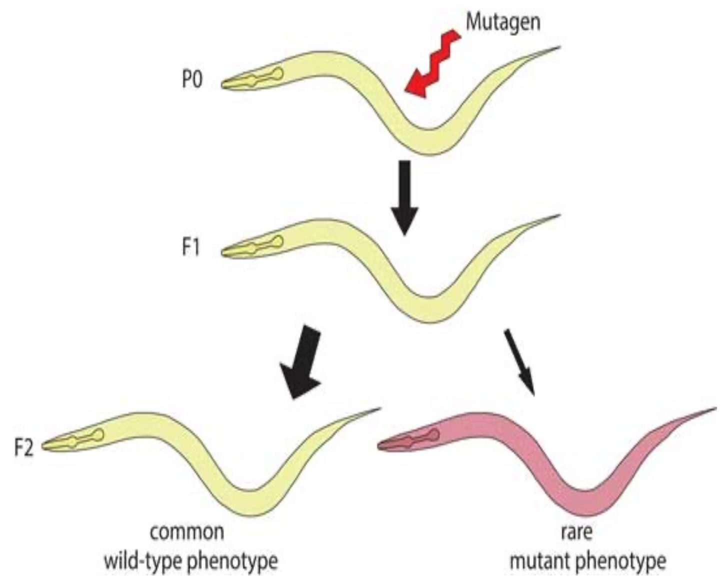
The gene *flp-13* encodes neuropeptides; is expressed in the sleep-promoting ALA neuron; and regulate sleep-like, quiescent behavior in *C. elegans*.

The *flp-13* transcript and protein are over-expressed in the presence of heat-inducible promoter (33° C). When *flp-13* is over expressed, the majority of the animals cease feeding and moving.

Mutagenesis: create random mutations of *flp-13* over-expressing transgenic animals by treating these animals chemically with ethyl methane-sulfonate.

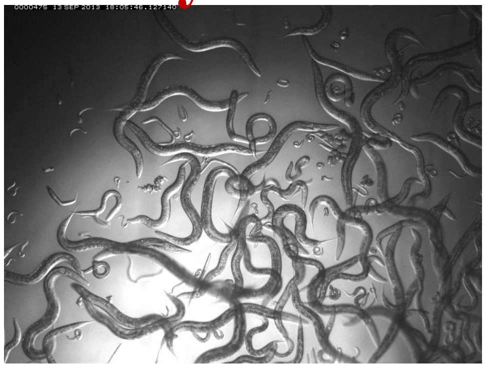


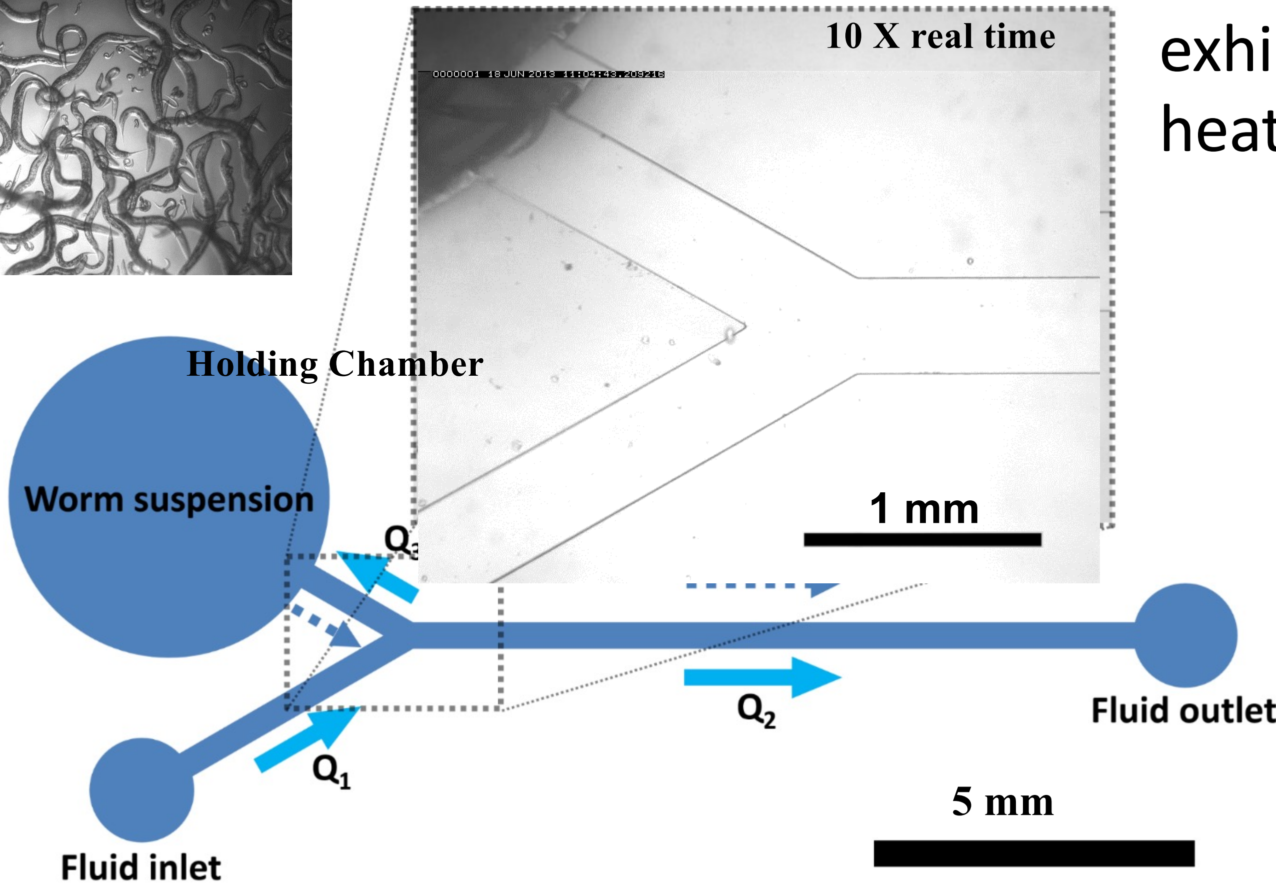
Animals with flp-13 cease to be active. Animals with flp-13 suppressor remain active

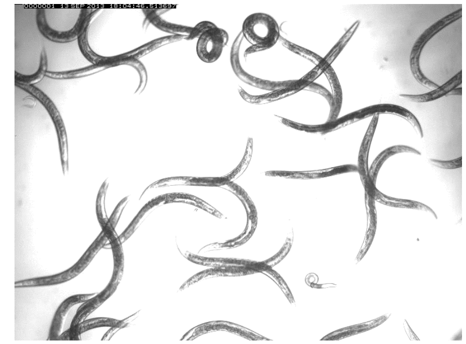


08:55

Motility-Based Nematode Sorter. Isolate granddaughters filial 2 (F2) of animals that exhibit motility after heat shock







number of worms sorted	# animals suspected to have suppressors gene	Assay time
210,800	12	8 h

DNA sequencing of the 12 sorted animals led to the identification of a previously unknown "*sleep-suppressing*" gene in all twelve isolated animals.

Yuan J, Zhou J, Raizen DM, Bau HH. High-throughput, motility-based sorter for microswimmers such as *C. elegans*. *Lab Chip*. 2015;15(13):2790-2798. doi:10.1039/c5lc00305a

Nematode Whisper

Worms sense their environment. For example, the worms sense the direction of an electric field and migrate towards the **negative** pole in a DC electric field (***electro-taxis***)

In our earlier experiments with motility assays, we took advantage of electro-taxis to control the worm's direction of motion.

Eventually, we discovered that the animals go against the flow without any inducement.

What is the mechanism that causes animals

to swim *against* the flow direction?

Is this trait deliberate or passive?

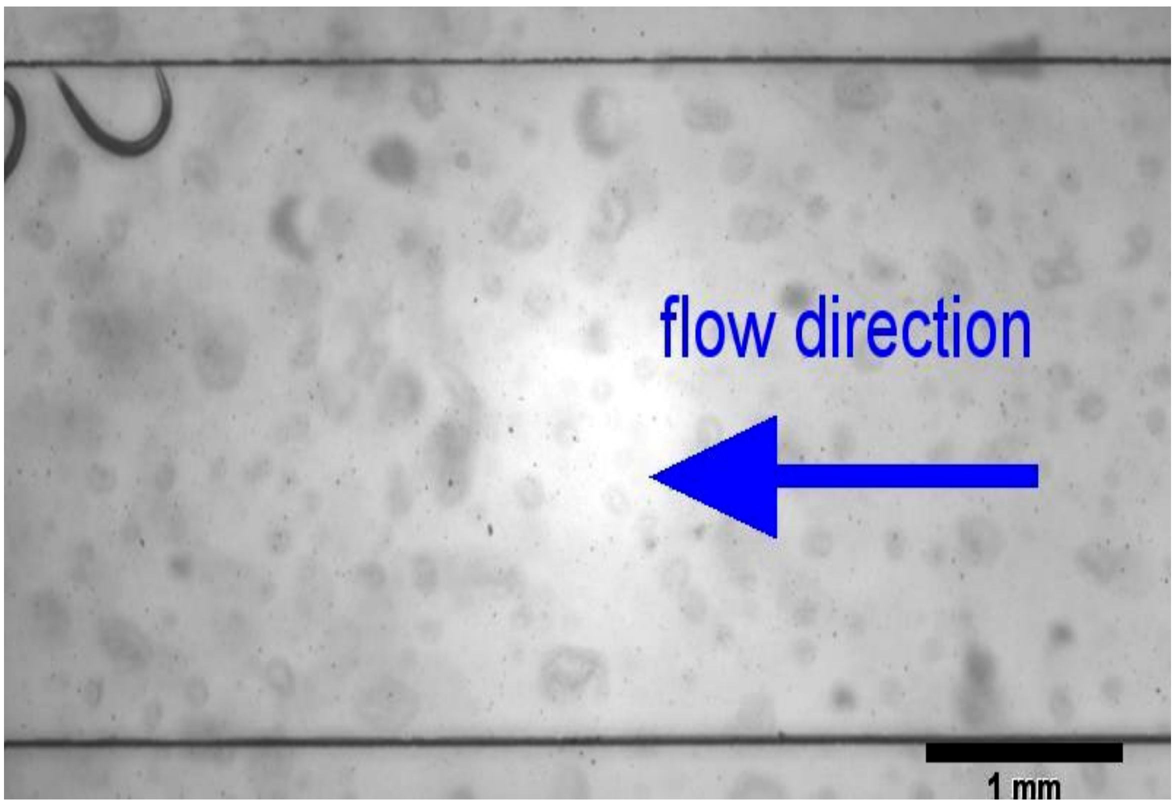
08:55

Rheotaxis-Propensity to Go Against the Flow

Various species, ranging from bacteria, sperm, nematodes, and zebra fish exhibit rheotaxis

There are conflicting reports in the literature whether nematodes exhibit rhoetaxis and the mechanisms are not known.

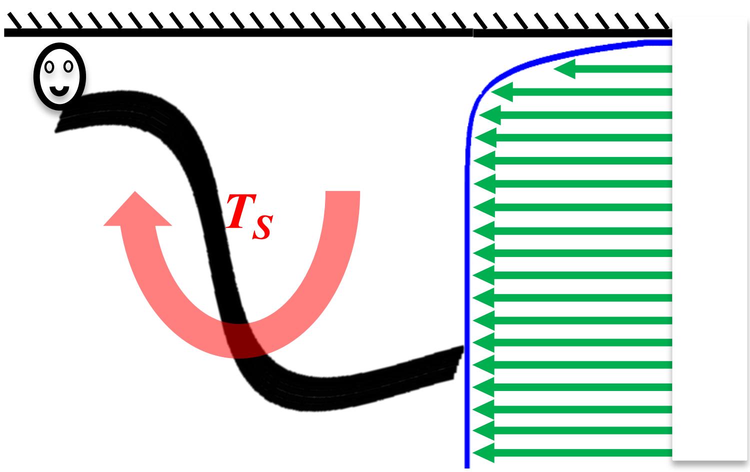
Our experiments indicate the *C. elegans* tends to swim against the flow

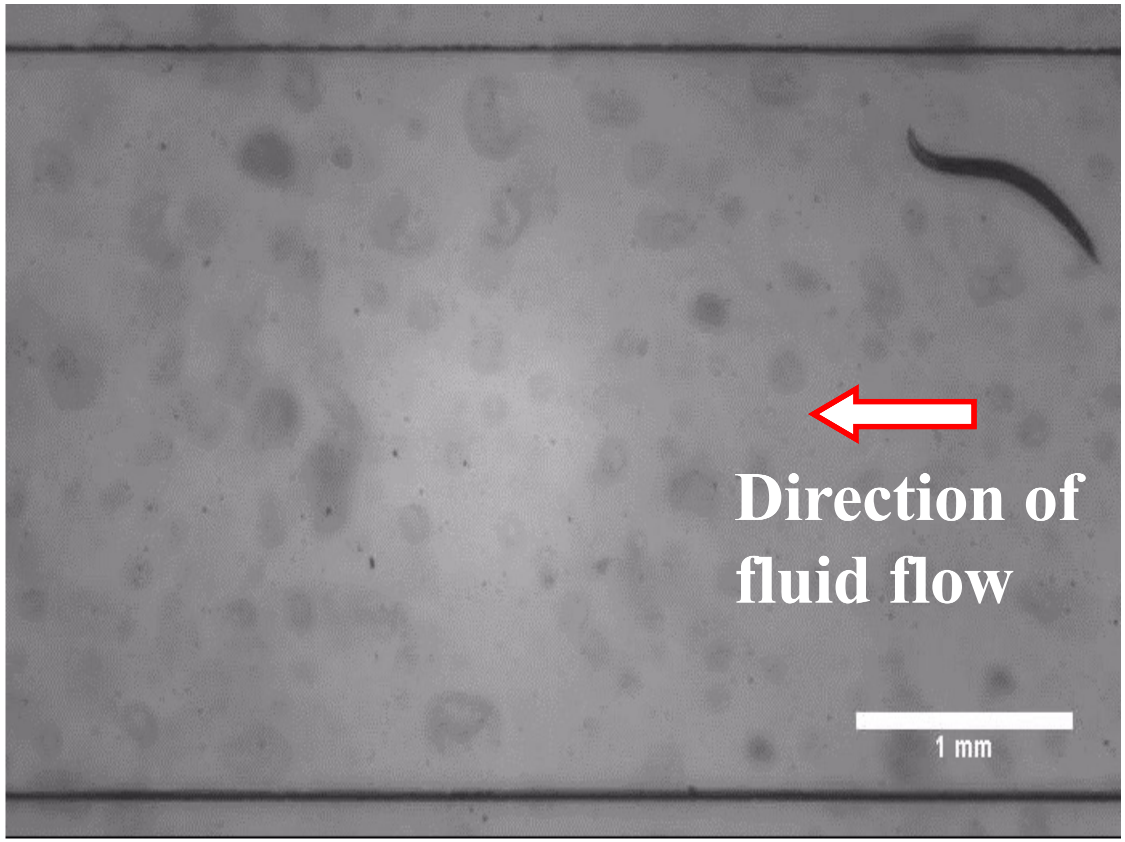


08:55

Yuan J, Raizen D., M, Bau H., H., *Proc Natl Acad Sci U S A* **112**, 3606-3611 (2015).

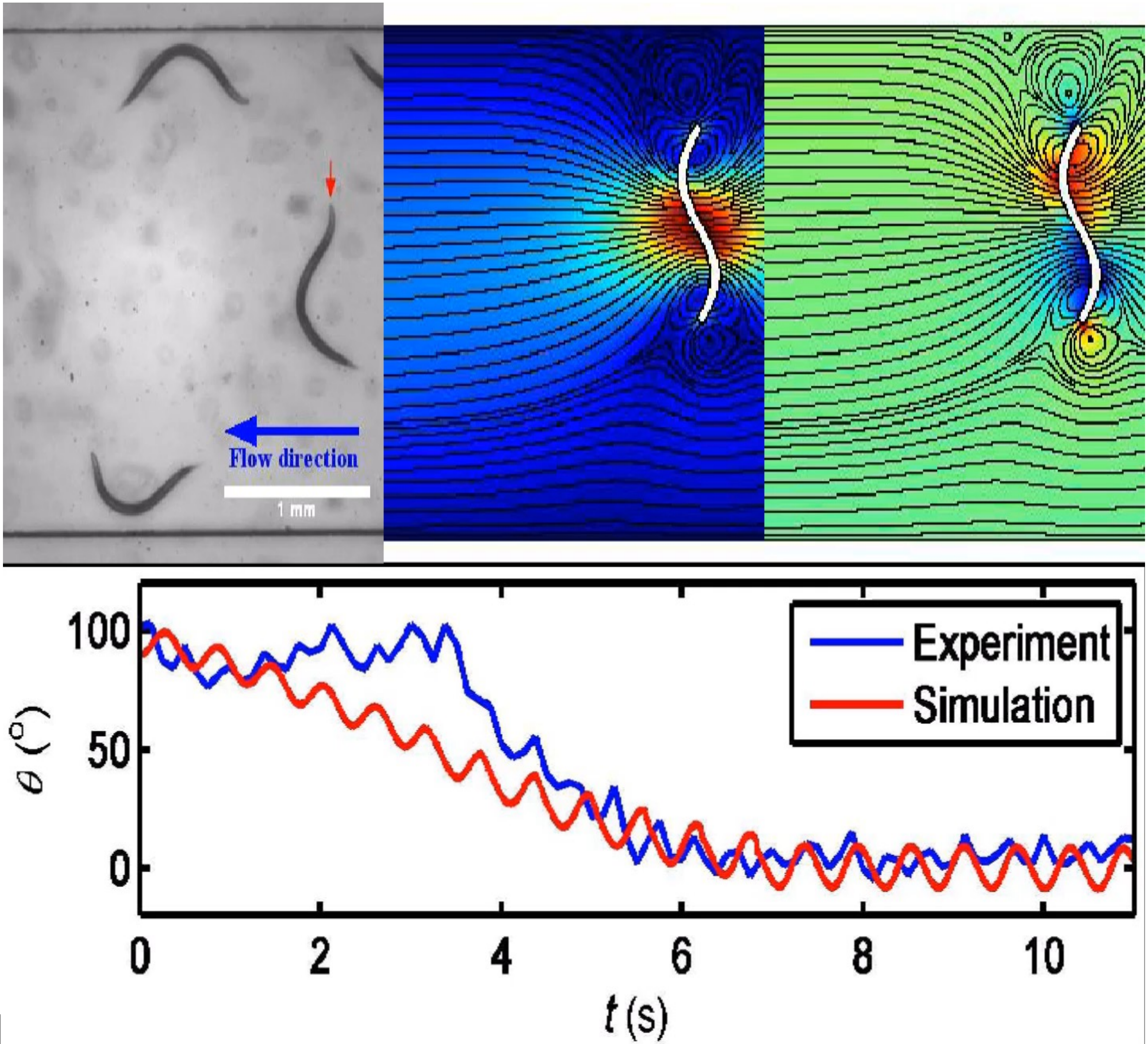
What causes nematodes to go against the flow?





Yuan J, Raizen D., M, Bau H., H, *Proc Natl Acad Sci U S A* **112**, 3606-3611 (2015).

08:55



Flow direction
1 mm
100
50
0
θ (°)
0
2
4
6
8
10
t (s)
Experiment
Simulation

08:55

Rheotaxis

Rheotaxis in undulatory swimmers is a consequence of hydrodynamics and does not involve sensory input from the animals.

The tendency to swim against the flow is common to many organisms, albeit the mechanisms involved may differ.

Rheotaxis may be beneficial to the animals as it allows them to maintain their positions in blood vessels, guts, and next to plants, avoiding being sweped with the flow

Our research suggests a therapeutic / disruptive means. It is enough to interfere with the animals’ motility without necessarily killing the animals to wash them out of the hosts’ bodies – less potent drugs.

Is rheotaxis important for humans?

08:55

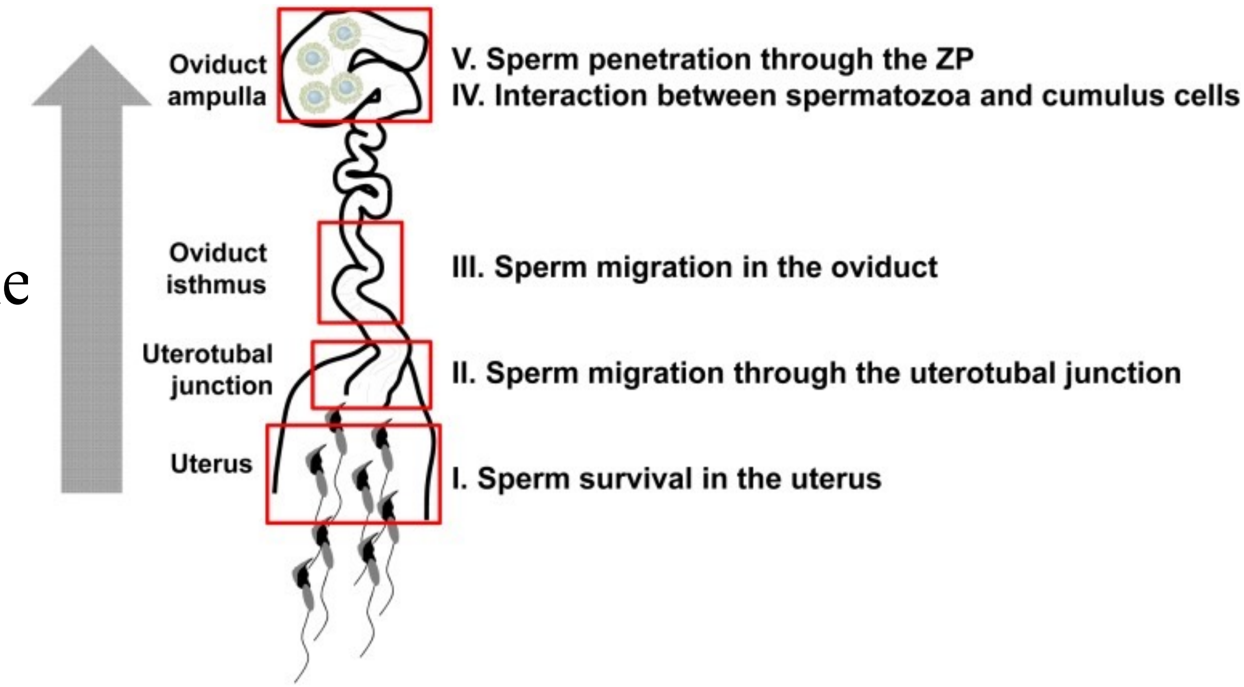
Mammalian Reproduction

Mammalian fertilization includes the critical step of sperm migration in the female reproductive tract.

The spermatozoa exhibits undulatory motion (like *C. elegans*).

But how do spermatozoa orient themselves in the oviduct in the presence of *adverse* oviductal film flow so that they swim against the flow?

Likely Answer: rheotaxis!



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5955741/

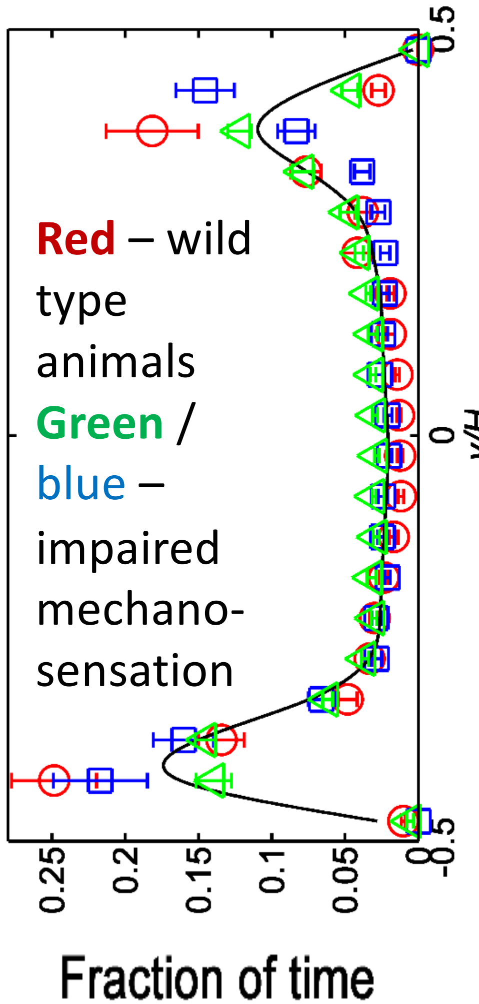
The spermatozoa flagellum's exhibits undulatory motion, enabling the sperm to swim

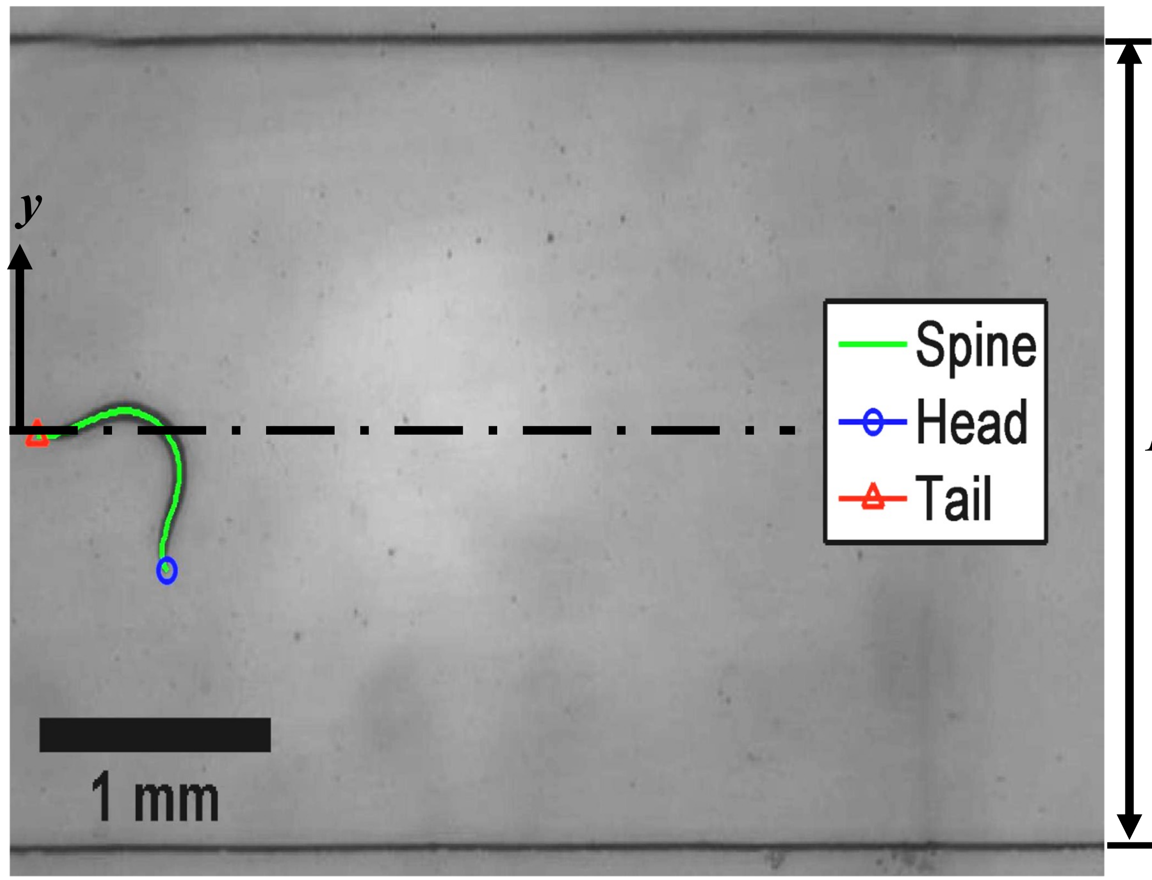
08:55

Attraction to Surfaces (border-taxis): **Experimental Observations**

Rheotaxis requires presence of velocity gradients –the animal must be close to a surface.

Residence time as a function of position along the conduit's width

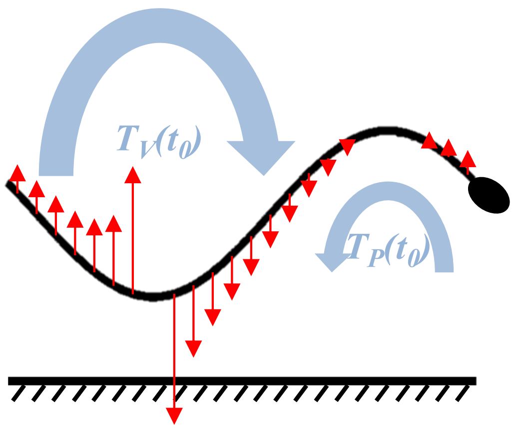




Yuan, J., Raizen, D. M., Bau, H. H., 2015, A hydrodynamic mechanism for attraction of undulatory microswimmers to surfaces (bordertaxis). Journal of the Royal Society Interface 12: 20150227. DOI: 10.1098/rsif.2015.0227

08:55

Attraction to Surfaces: Short-range Hydrodynamic Rotation

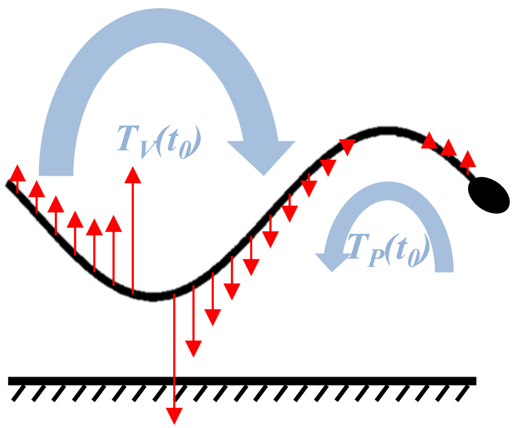


Prescribed swimming gait:

$$y_s = h + b\sin\frac{2\pi}{\lambda}(\mathrm{x} + U_w\,\mathrm{t}), \quad (0 \le x \le \lambda)$$

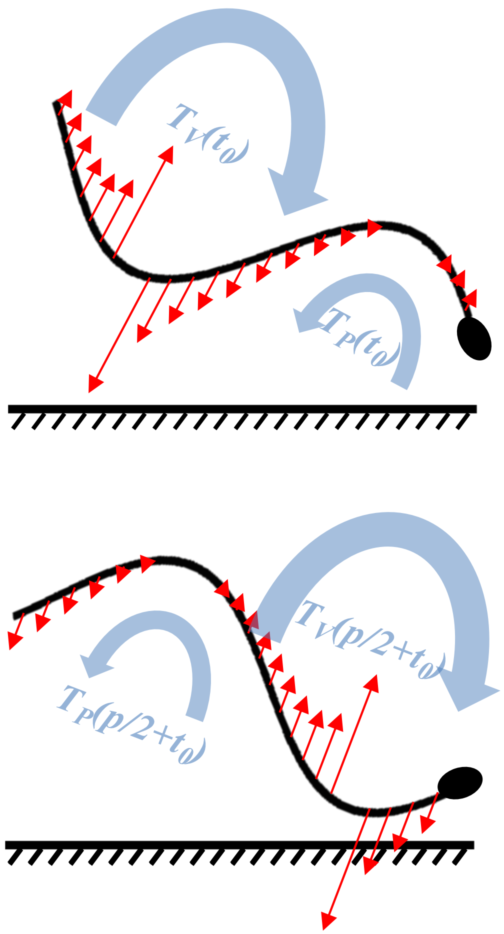
08:55

Attraction to Surfaces: The Short-range Hydrodynamic Effect



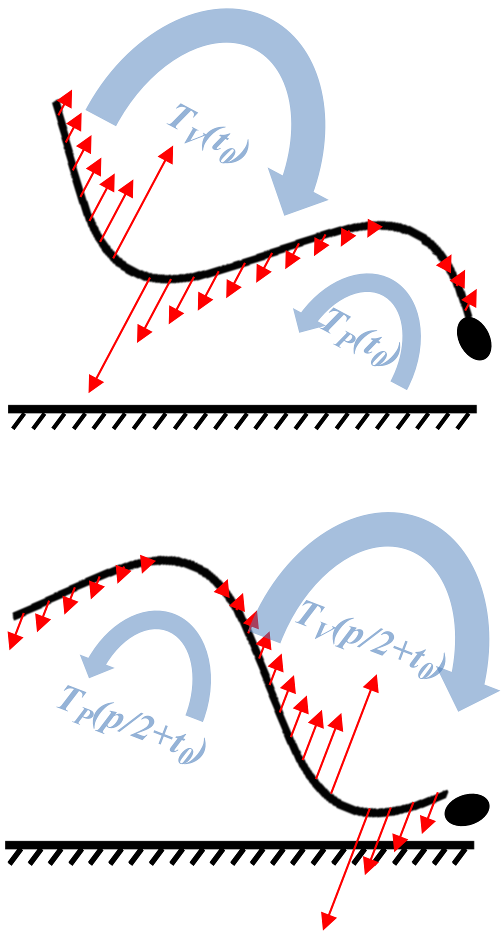
08:55

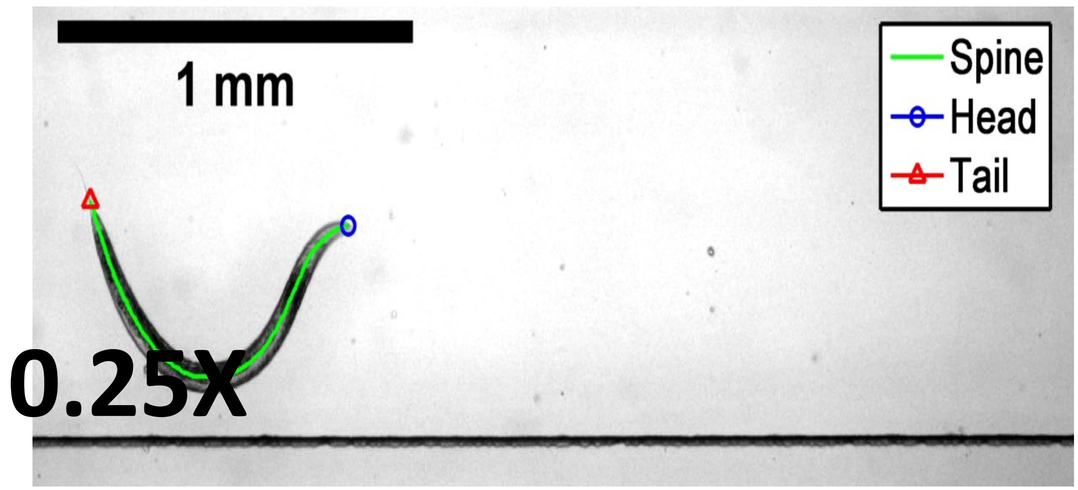
Attraction to Surfaces: The Short-range Hydrodynamic Effect



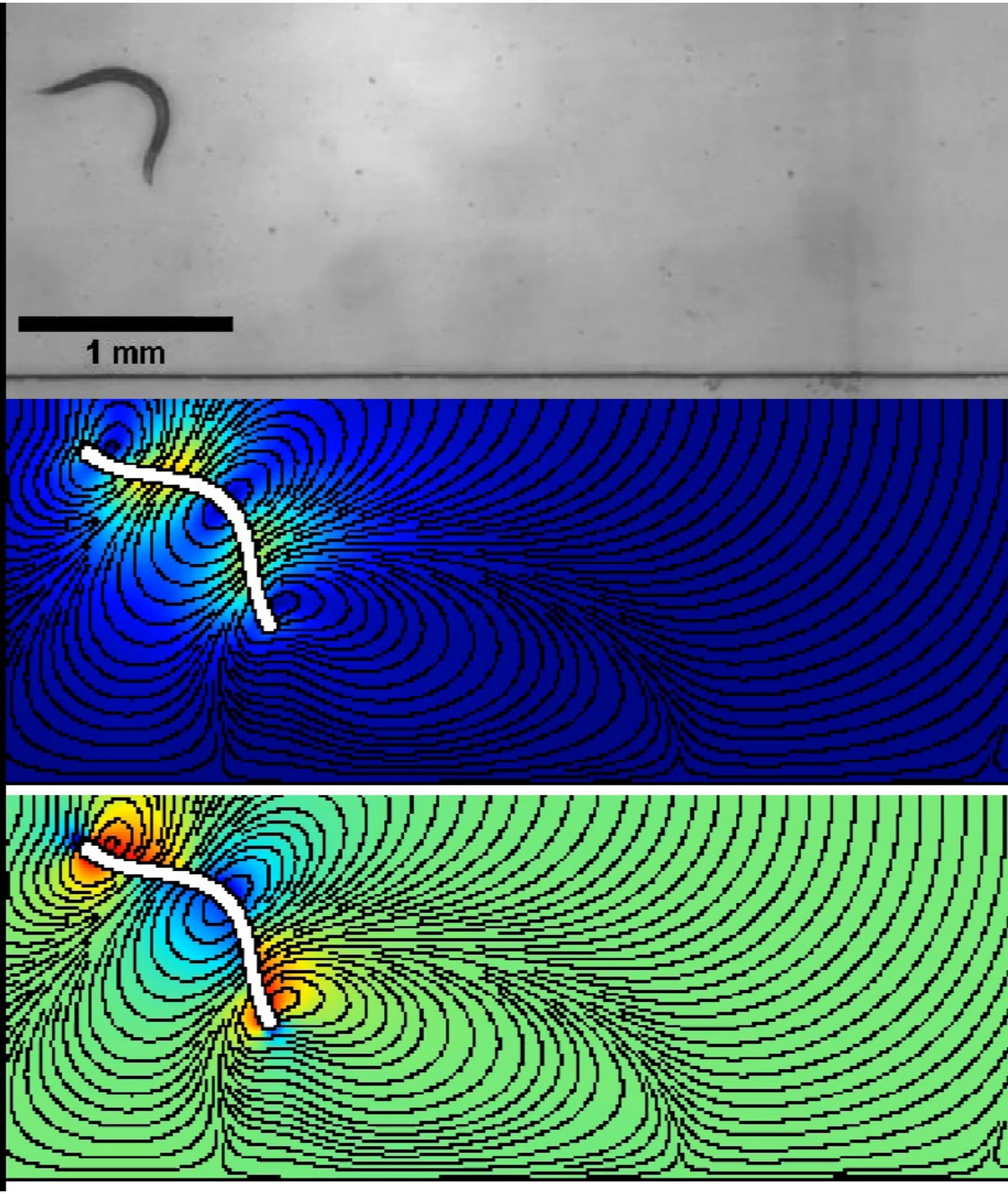
08:55

Attraction to Surfaces: The Short-range Hydrodynamic Effect

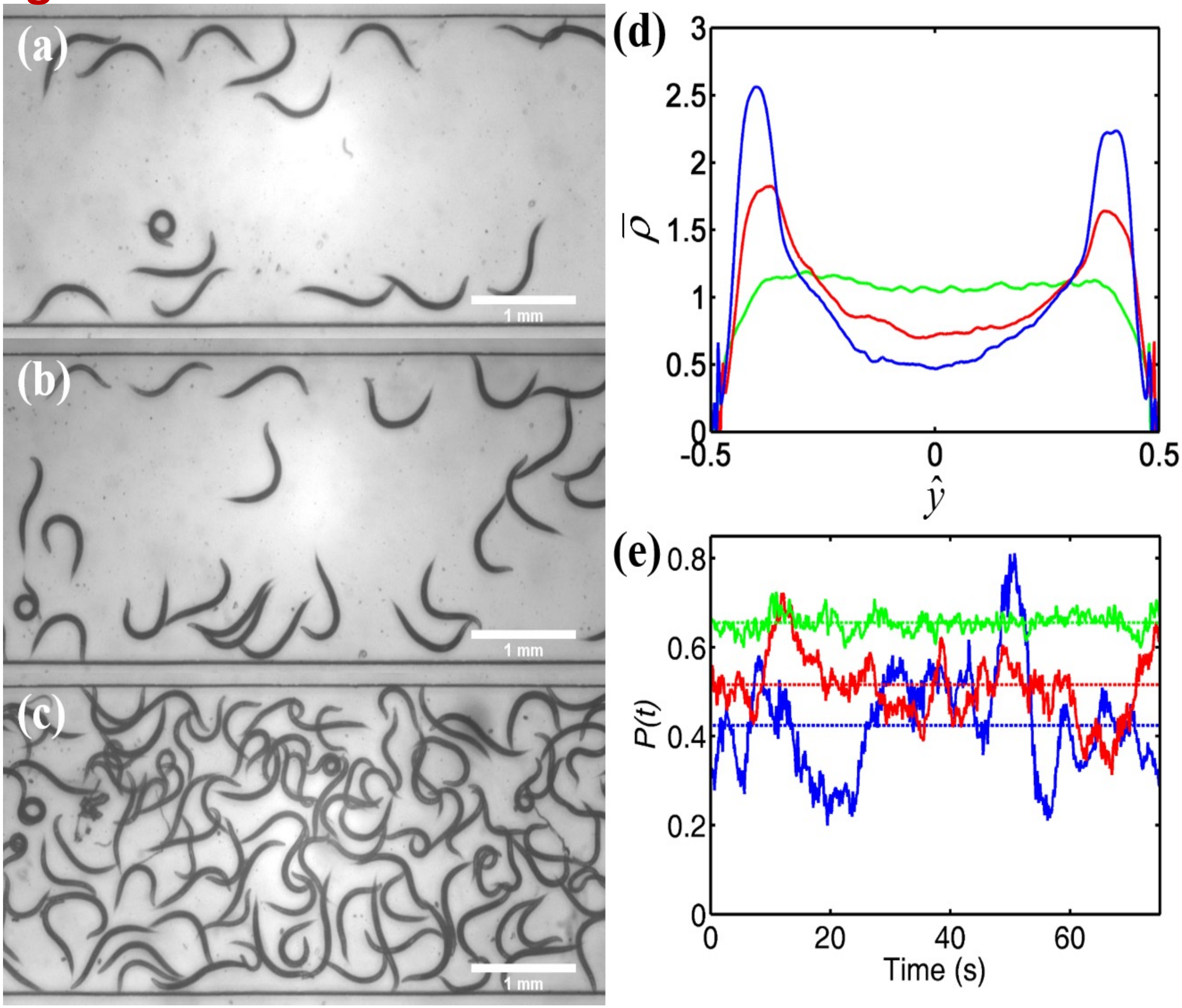




08:55


1 mm

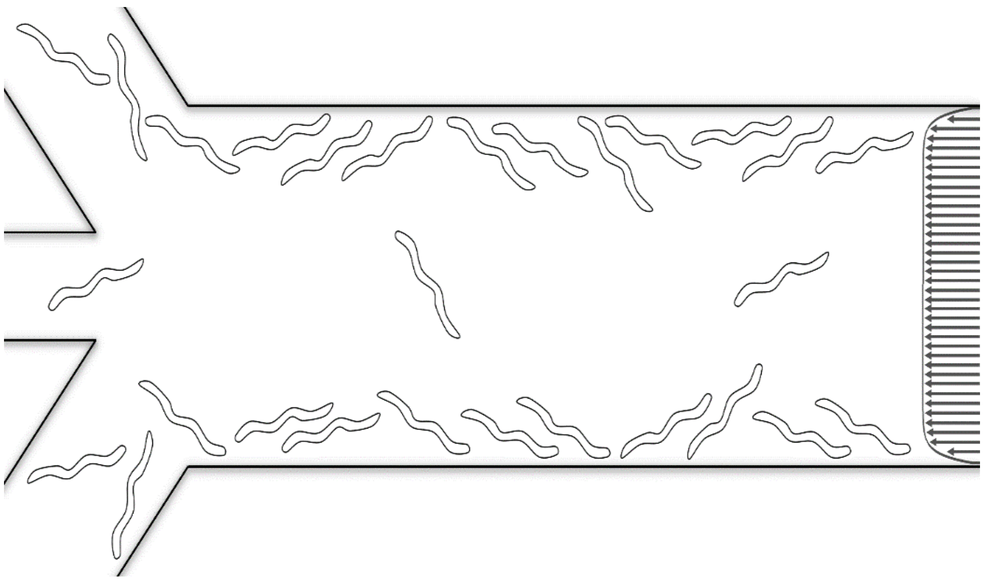
Aggregation next to Surfaces

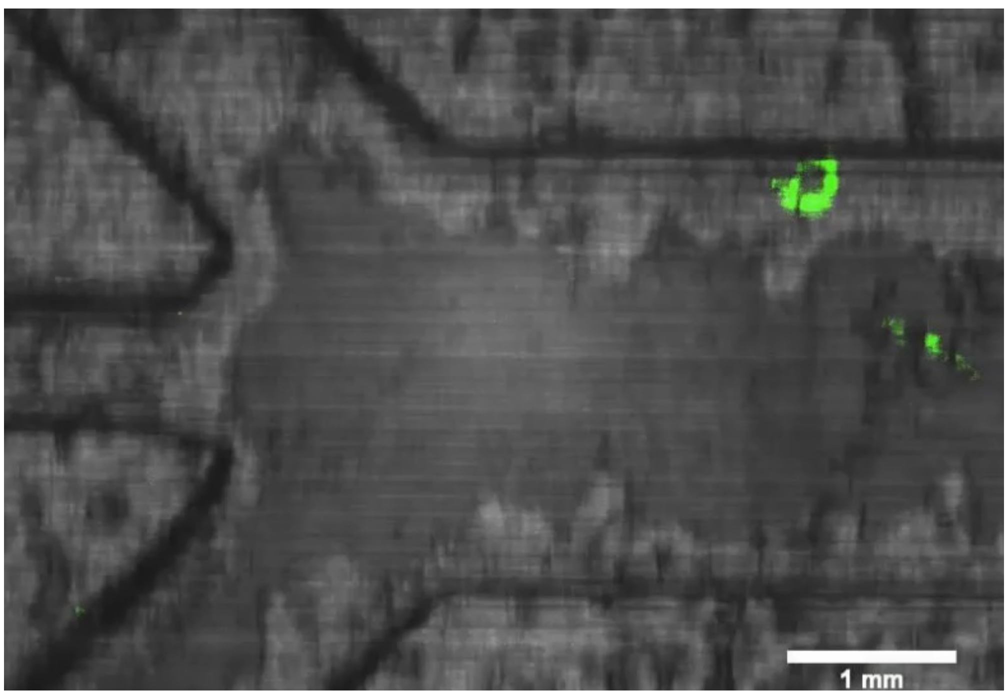


08:55

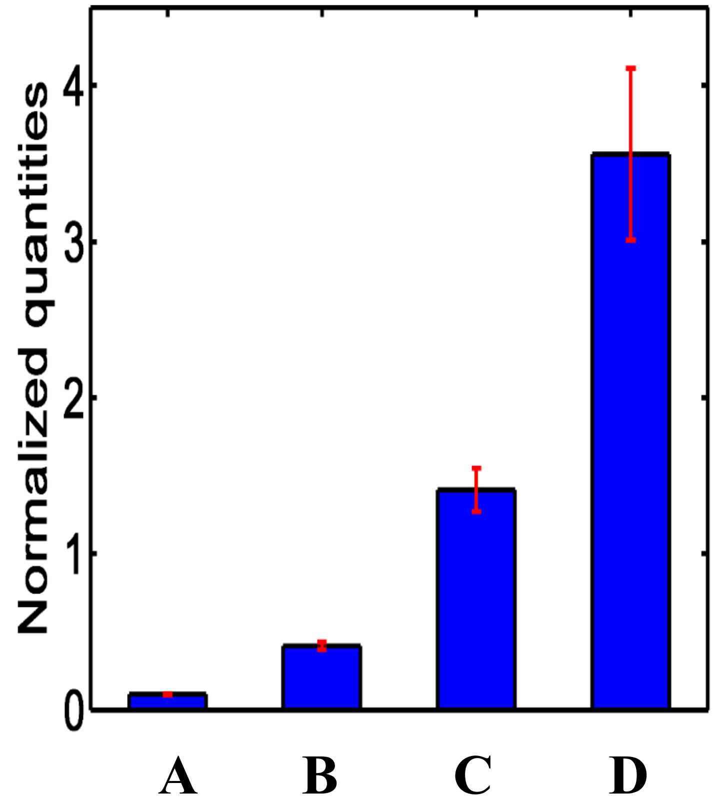
In the dilute limit, nematodes aggregate next to the surfaces. At high concentration, the nematode distribution is nearly uniform.

Taking advantage of surface attraction: The Worm Skimmer





Data for the central branch



A: Fraction of active *C. elegans* collected.
B: The normalized concentration of active *C. elegans*.
C: The normalized concentration of inactive *C. elegans*.
D: The normalized inactive/active *C. elegans* ratio.

BORDER-TAXIS

The propensity to attract to surfaces is of hydrodynamic origins and does not rely on sensory/neural input.

As a result of attraction to surfaces, undulatory swimmers aggregate next to surfaces.

Surface proximity is beneficial to the animals as it increases the probability of penetration into a host (*e.g.*, parasitic worms), maintains animals in regions rich in nutrients, and assist in navigation and mating.

One can take advantage of surface attraction to design microfluidic systems for nematode sorting. Designers of microfluidic systems must account for surface attraction as it may interfere with other traits such as chemotaxis.

Surface attraction facilitates rheotaxis.

Yuan, J., Raizen, D. M., Bau, H. H., 2015, A hydrodynamic mechanism for attraction of undulatory micro-swimmers to surfaces (bordertaxis). Journal of the Royal Society Interface 12: 20150227.   DOI: 10.1098/rsif.2015.0227

08:55

How does C. elegans remain "afloat"?

C. elegans is *heavier* than water (1.07 g/ml)!

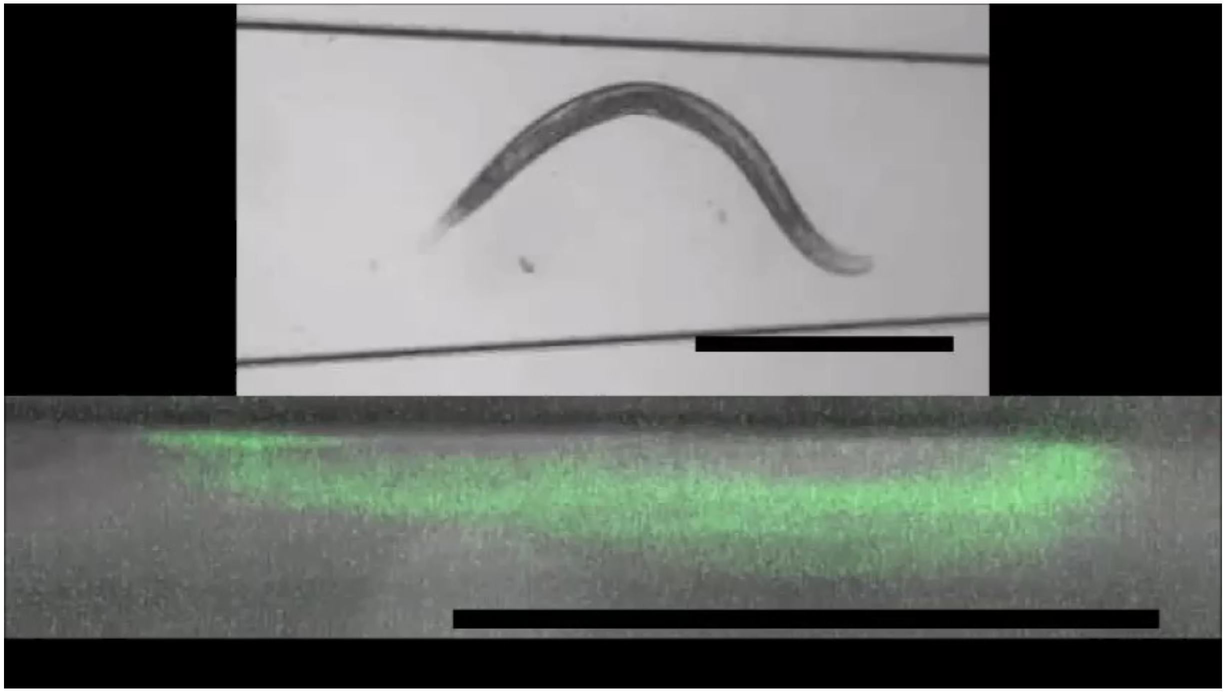
Yet, the animal exhibits swimming – like behavior (in contrast to crawling on the bottom surface).

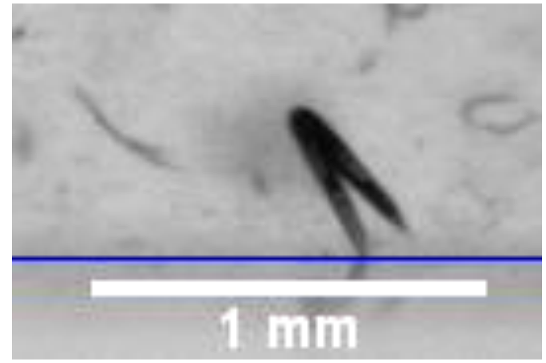
Questions:

Does *C. elegans* sense gravity and adjust its swimming angle to counteract gravity?

08:55

C. elegans sinks to the bottom and swims along the floor using a bump (collisions) and undulate (up-and-down) strategy



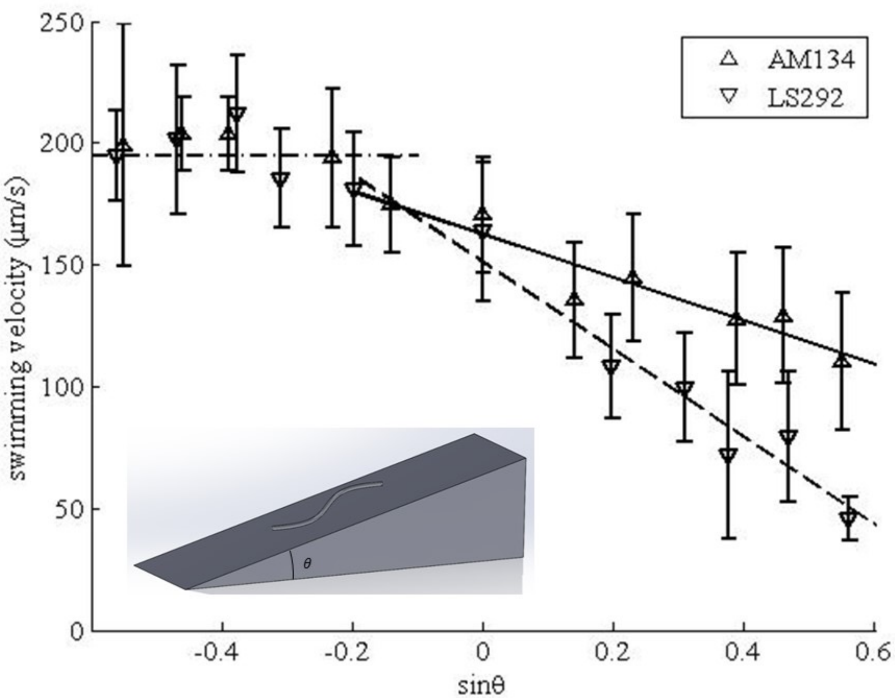


C. elegans propel along a surface while colliding with the bottom surface.

08:55

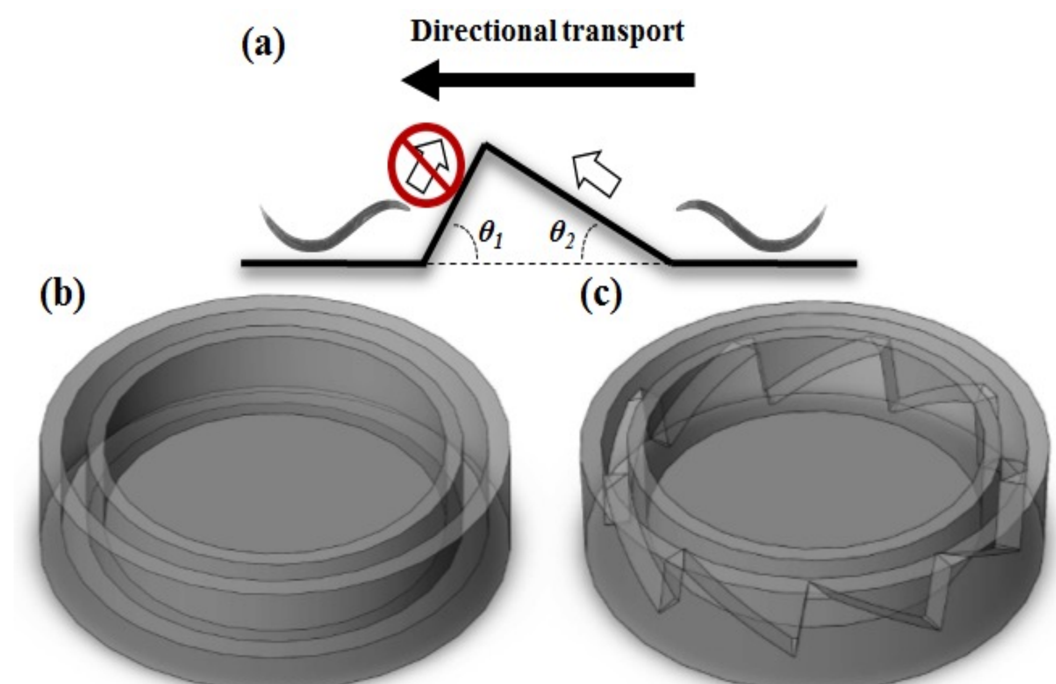
Surface Following: In the presence of a wedge the animal swims along the wedge

The wedge provides a method to estimate propulsive thrust based on stall angle



Propulsive-power based sorting

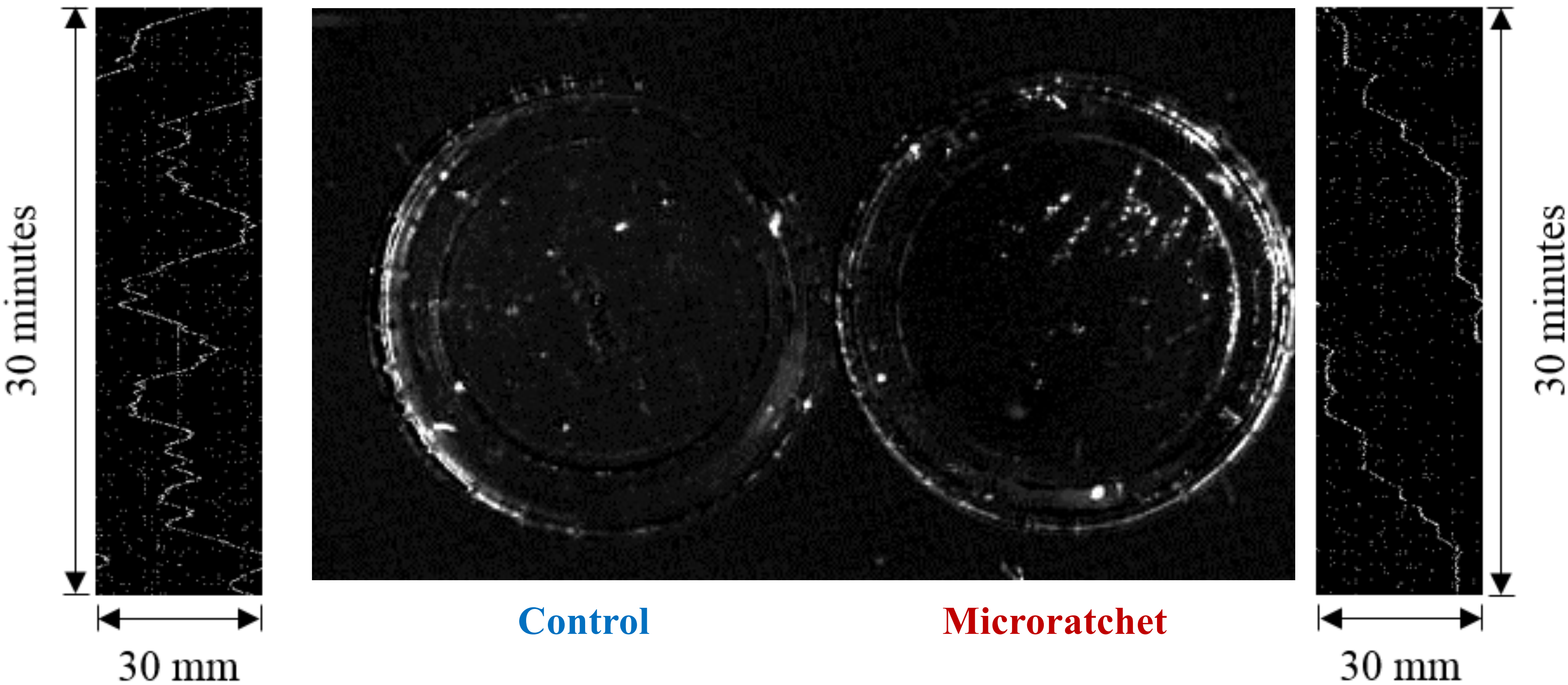
The nematode ratchet



08:55

Yuan, J., Ko, H.,Raizen, D., Bau, H., H., 2016, Terrain Following and Applications: Caenorhabditis elegans Swims along the Floor using a Bump and Undulate Strategy, Proc Royal Society Interface. Volume 13, issue 124, DOI: 10.1098/rsif.2016.0612.

The Worm Micro-Ratchet



If the ratchet were placed on low friction bearing, the worms may be able to rotate the ratchet.

Yuan, J., Ko, H.,Raizen, D., Bau, H., H., 2016, Terrain Following and Applications: Caenorhabditis elegans Swims along the Floor using a Bump and Undulate Strategy, Proc Royal Society Interface. Volume 13, issue 124, DOI: 10.1098/rsif.2016.0612.

08:55

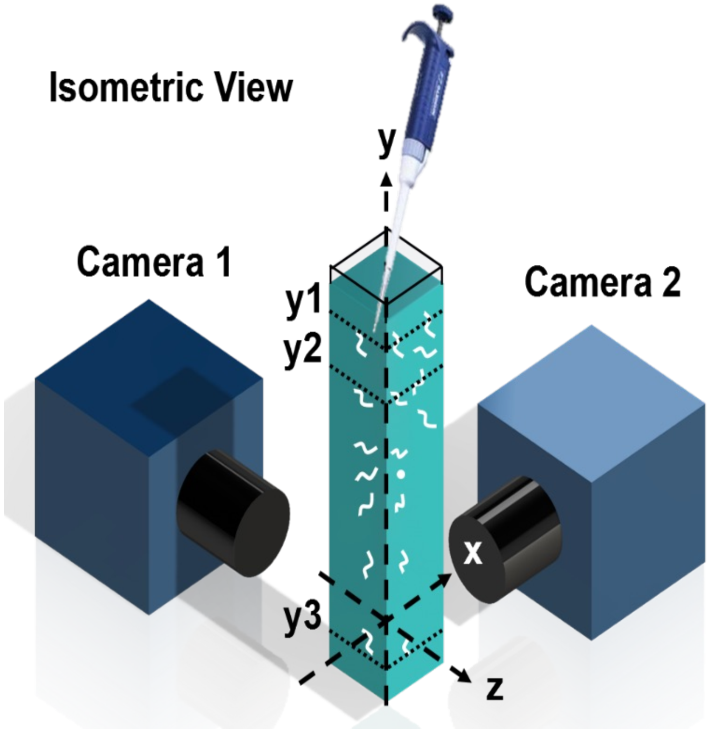
BUMP and SWIM

The worms sink to the bottom and use collisions to interact with the terrain to remain afloat. This mechanism does not require gravity sensing.

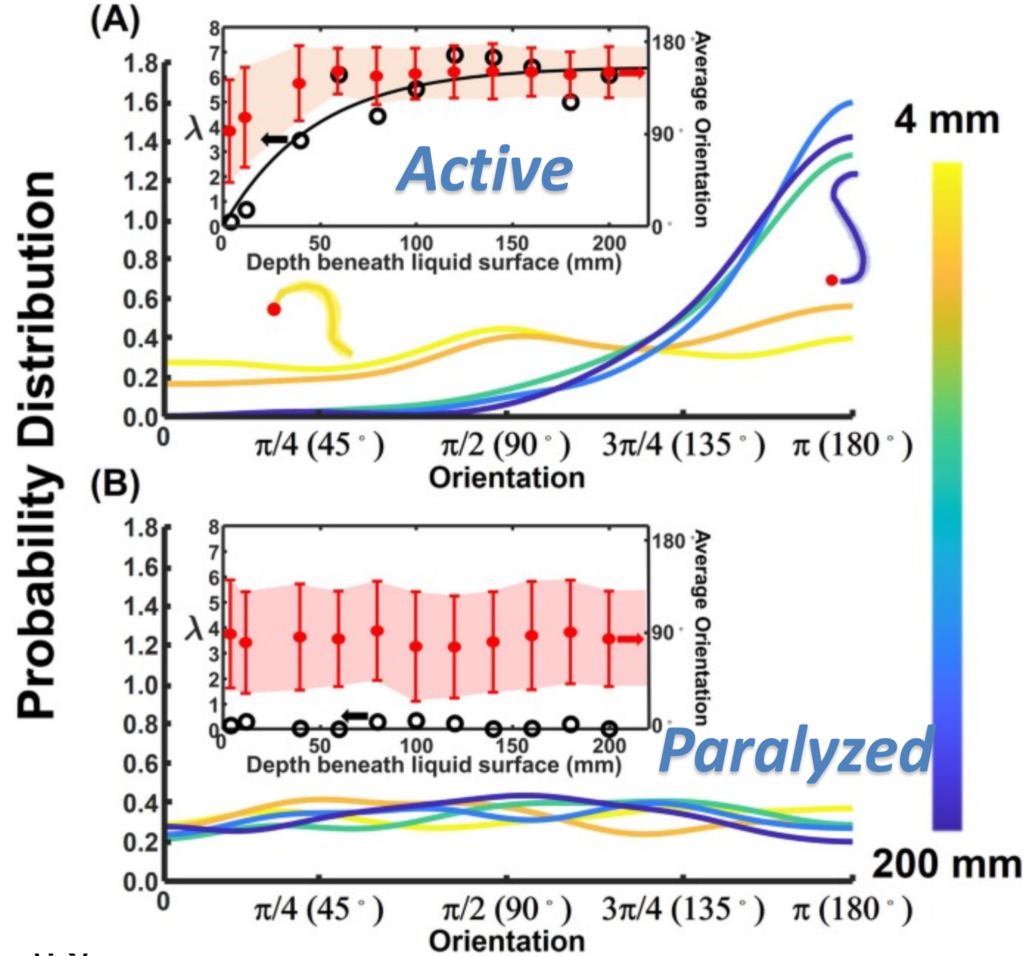
The question whether *C. elegans* sense and react to gravity is still open.

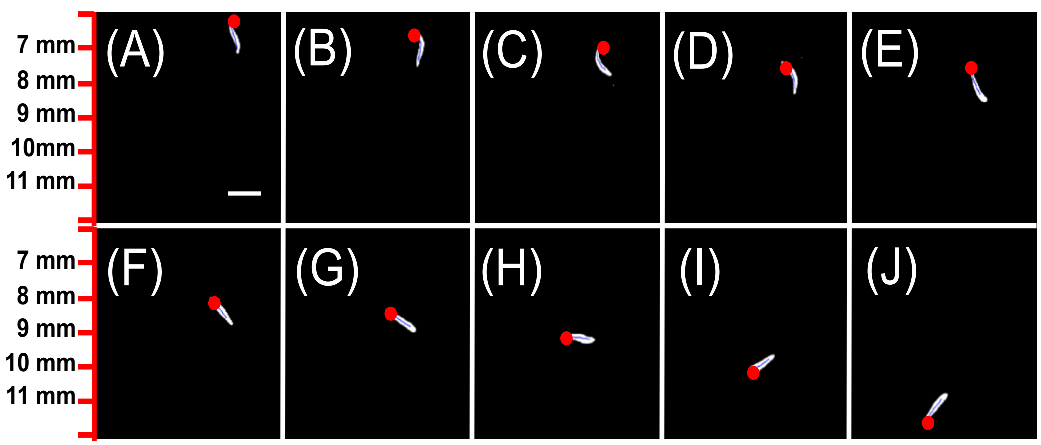
08:55

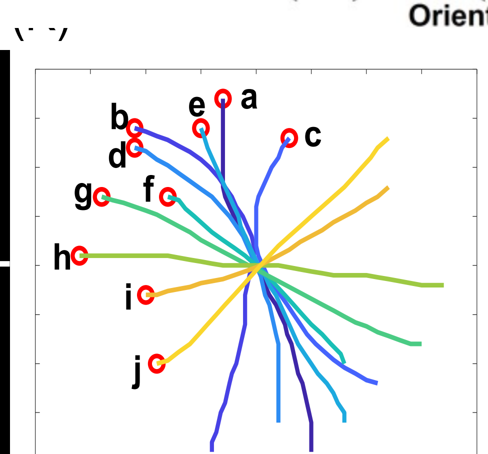
C. elegans exhibits gravitaxis



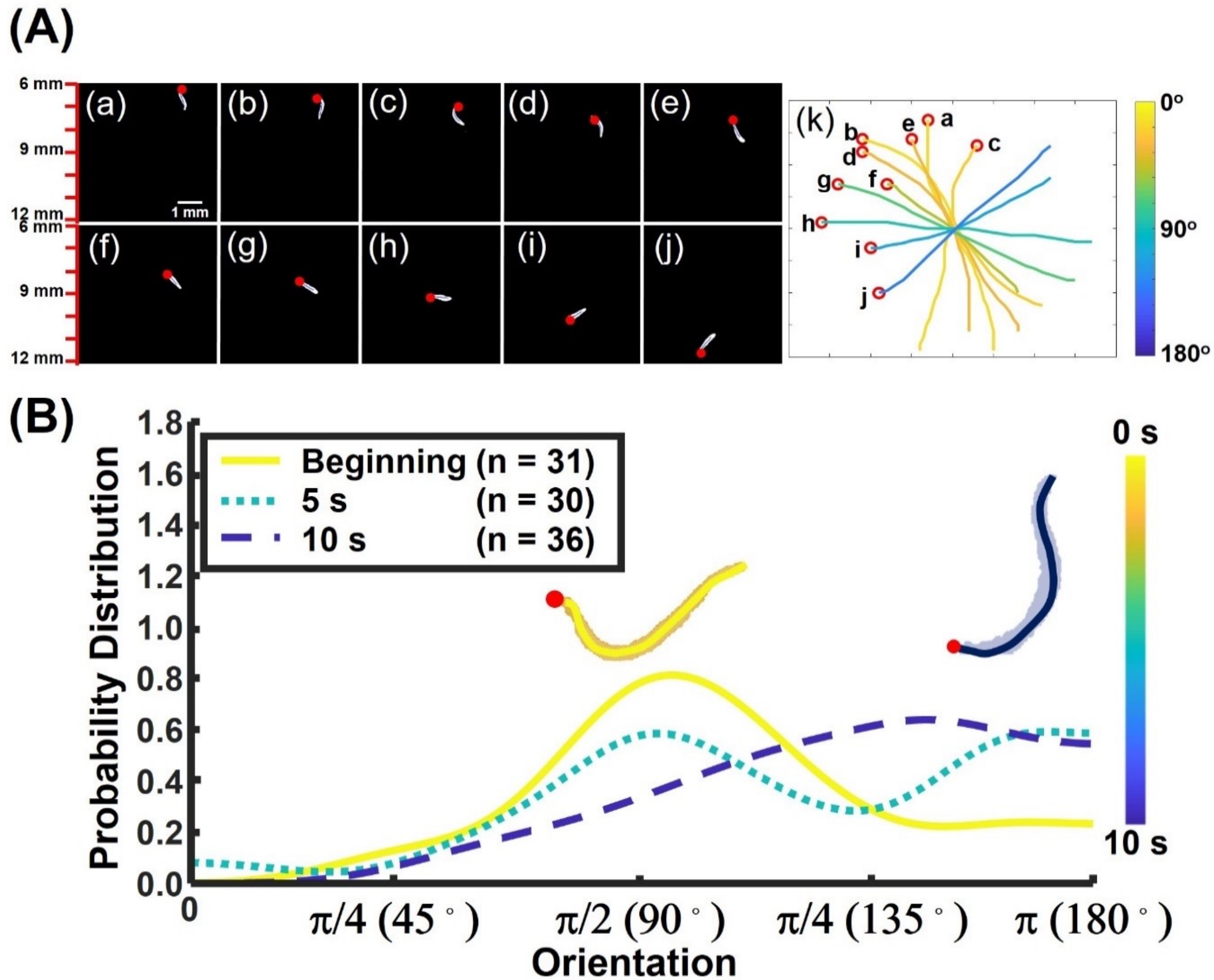
$$f(\theta) = \frac{\lambda}{2 Sinh \lambda} e^{\lambda \cos(\pi - \theta)} \sin(\pi - \theta)$$







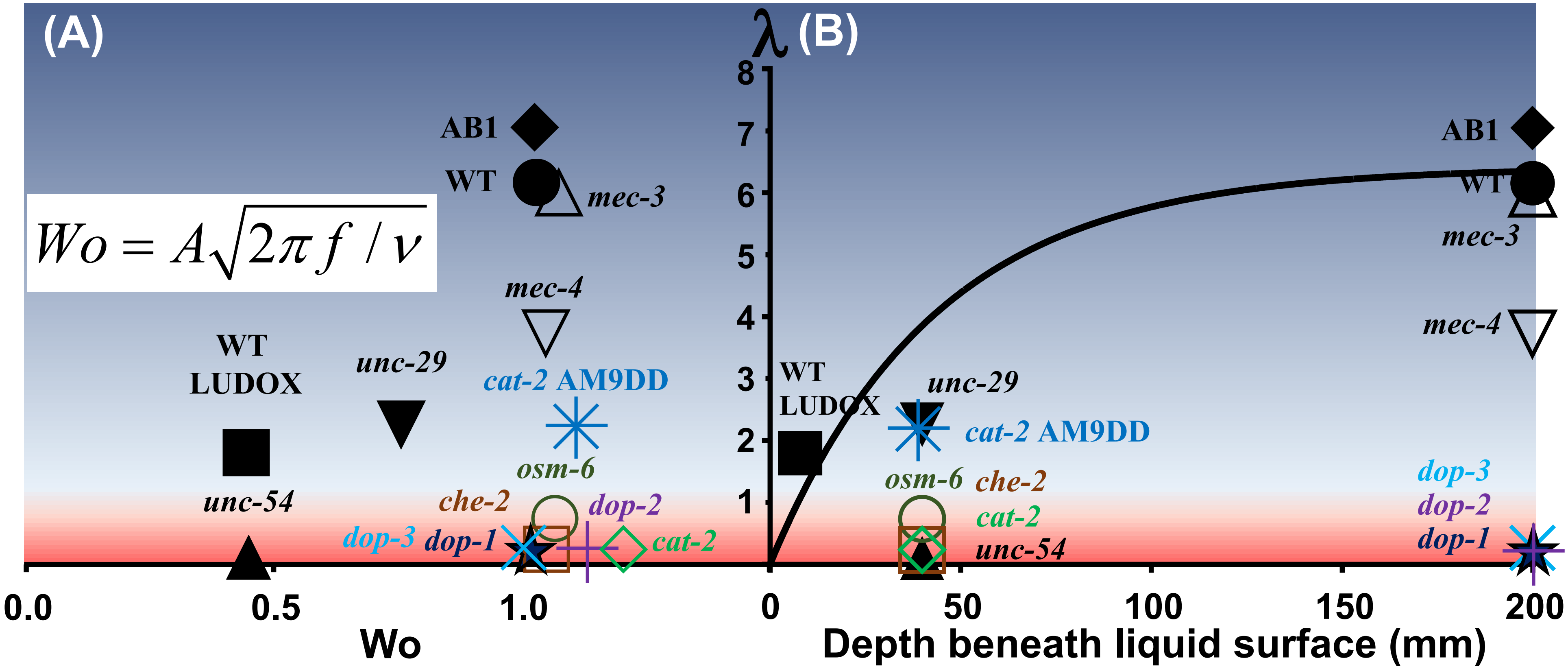
08:55



Wild-type animals rotate to align their direction of motion downward when suspended in a solution denser than the animals.

Chen WL, Ko H, Chuang HS, Raizen DM, Bau HH. Caenorhabditis elegans exhibits positive gravitaxis. BMC Biol. 2021 Sep 14;19(1):186. doi: 10.1186/s12915-021-01119-9. PMID: 34517863; PMCID: PMC8439010

08:55



Animals' propensity to gravitax (λ) as a function of animal vitality (Wo) (**A**) and the animal's depth beneath the liquid surface (**B**). The reddish region (λ<1) indicates absence of gravitaxis and an orientational probability distribution (*pdf*) that is dissimilar to that of wild-type animals with confidence level >95%. The bluish region indicates competency to gravitax (λ>1). The residence time of animals in LUDOX was converted to equivalent depth with the aid of the settling velocity

08:55

Chen WL, Ko H, Chuang HS, Raizen DM, Bau HH. Caenorhabditis elegans exhibits positive gravitaxis. BMC Biol. 2021 Sep 14;19(1):186. doi: 10.1186/s12915-021-01119-9. PMID: 34517863; PMCID: PMC8439010

Gravitaxis

In aqueous solutions, motile WT *C. elegans* align their swimming direction with the gravity vector while immobile worms do not.

The worms orient downward regardless of whether they are suspended in a solution less dense (downward sedimentation) or denser (upward sedimentation) than themselves.

Gravitaxis is minimally affected by the animals' gait but **requires sensory cilia, dopamine neurotransmission**, and **motility**; it does not require genes that function in the body touch response.

Lack of gravitaxis in dopamine deficient animals is pharmaceutically recoverable.

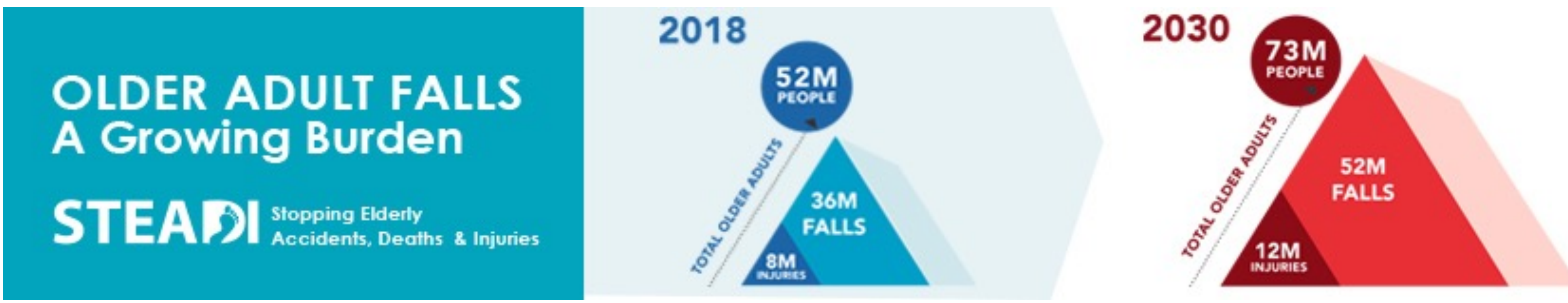
Gravitaxis is not passively mediated by such as non-uniform mass distribution or hydrodynamic effects. It requires active neural processes.

C. elegans provides a genetically tractable system to study molecular and neural mechanisms of gravity sensing.

Implication to Human Health

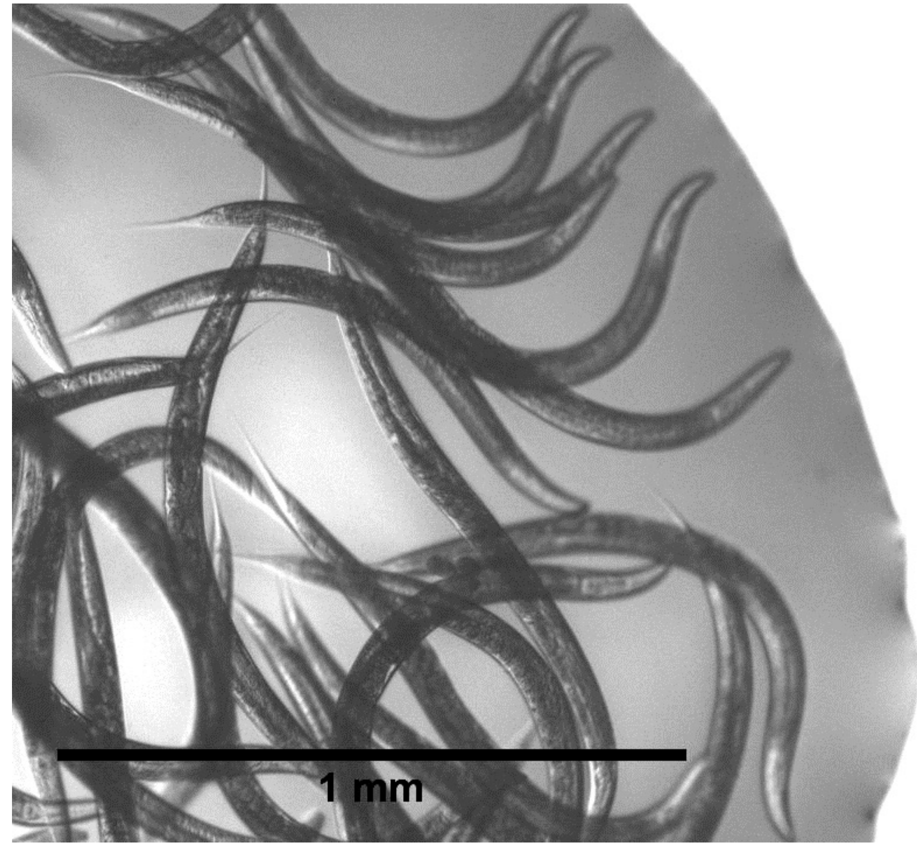
In the USA, the annual medical cost of falls among the elderly (>65) exceeds **$50** billion with significant additional cost in non-medical care, lost productivity, and significant reduction in quality of life.

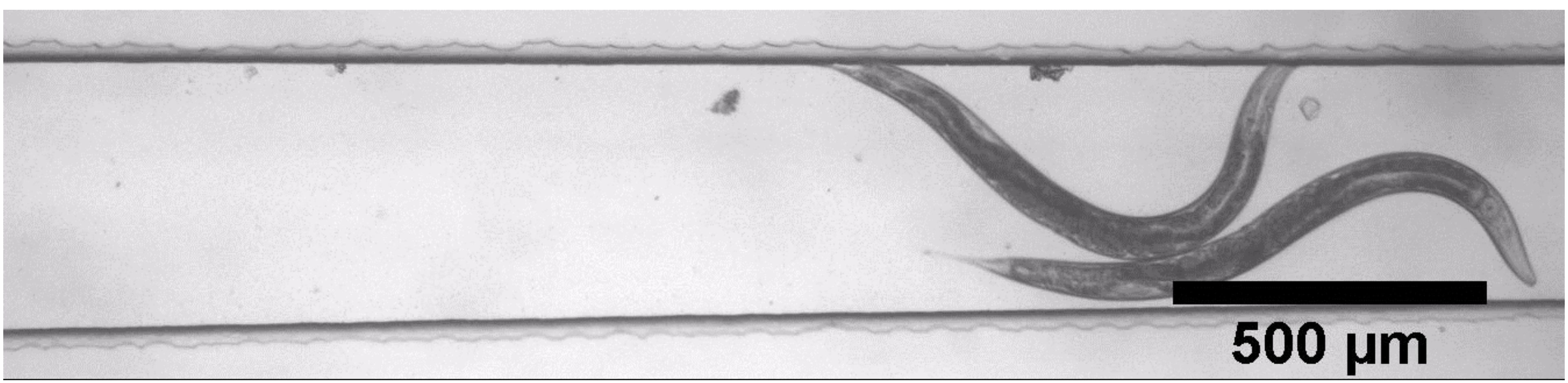
Better understanding of the molecular mechanisms of gravity sensing may suggest new therapies.



https://www.cdc.gov/falls/data/fall-cost.html#:~:text=Falls%20among%20adults%20age%2065,spent%20related%20to%20fatal%20falls.

Active Suspensions
COLLECTIVE BEHAVIOR
GAIT SYNCHRONIZATION

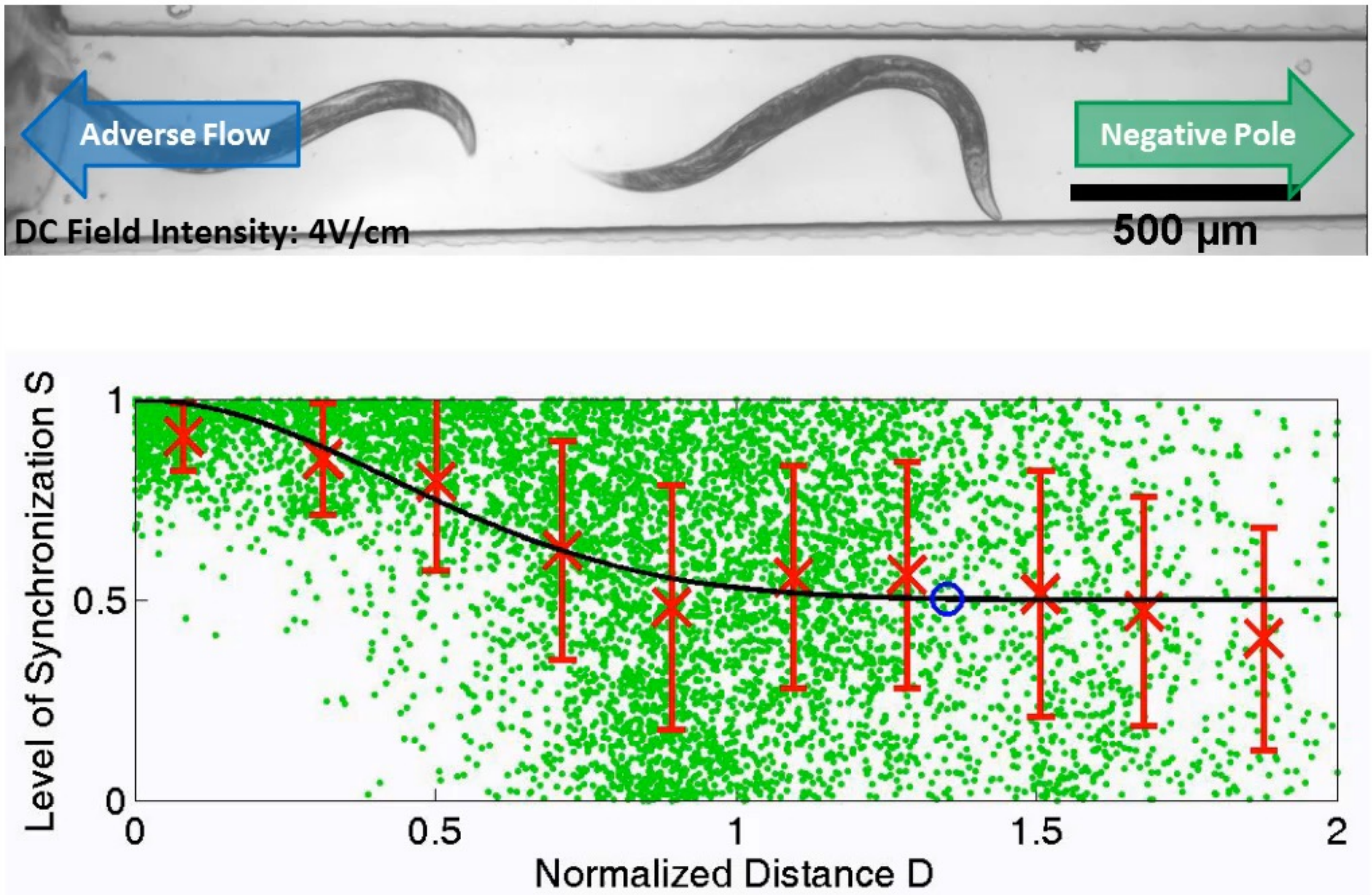




08:55

Yuan, J., Raizen, D., and Bau, H. H., 2014, Gait Synchronization in Caenorhabditis *elegans*, PNAS 111 (19), 6865–6870. doi: 10.1073/pnas.1401828111.

$$S(t) = 1 - \frac{2}{\lambda}\min\left(Mod\left(d(\mathrm{t}),\lambda\right), \lambda - Mod\left(d(\mathrm{t}),\lambda\right)\right)$$



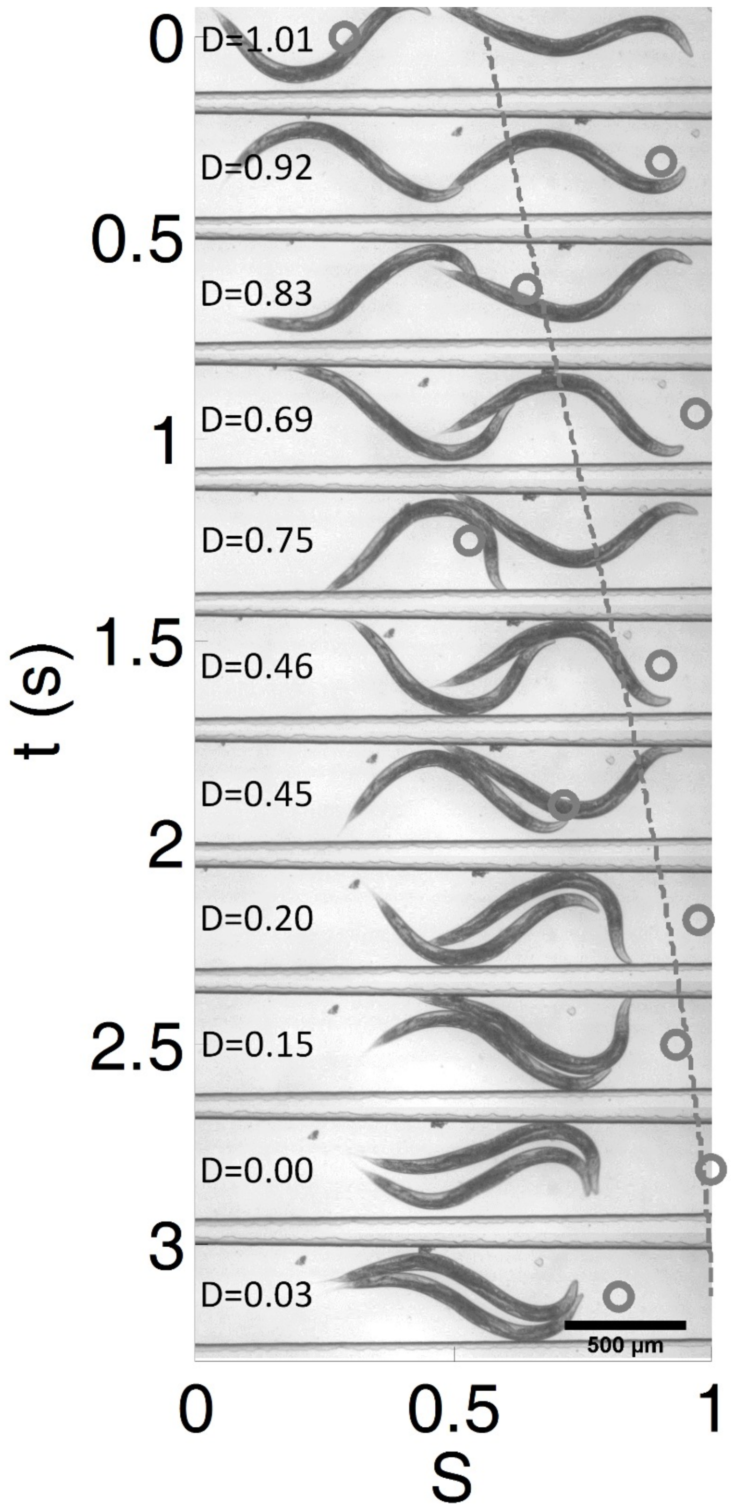
08:55

Yuan, J., Raizen, D., and Bau, H. H., 2014, Gait Synchronization in Caenorhabditis *elegans*, PNAS 111 (19), 6865–6870. doi: 10.1073/pnas.1401828111.

When confined, undulatory swimmers synchronize their gait to avoid jamming and to optimize the use of space.

Synchronization is brought about through steric hindrance (collisions) and does not require sensory input.

Animals deficient in mechano-sensation behave just like their wild type counterparts.



08:55

SUMMARY

Worms constitute a significant fraction of animal species and are involved in diverse ecosystems. It is important to understand the interactions of worms with their environment to minimize adverse effects such as caused by parasitic worms.

Of particular interest to us is the model animal *C. elegans* that has a simple nervous system, is easy to cultivate, duplicates many of the genes present in mammalian animals, including humans, and in parasitic worms and is amenable for discovering relationships between genotype and phenotype.

Microfluidics enables high throughput motility assays to examine the effects of age, exercise, drugs, and chemicals, among other things, on animal behavior

Although worms have a nervous system, certain traits such as boundary attraction, rheotaxis, and gait synchronization are caused by hydrodynamic forces and do not require sensory input.

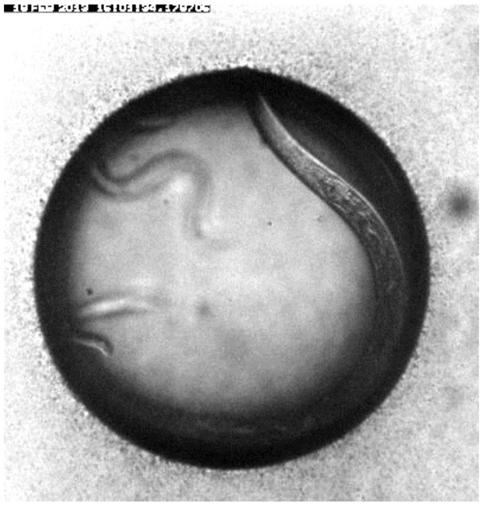
Worms, like molecules, rely on collisions among themselves and with surfaces to adjust their behavior.

C. elegans senses and reacts to gravitational fields and to electric fields. Gravitaxis requires ciliated cells and dopamine. Understanding the molecular mechanisms of gravitaxis may help address loss of balance in the elderly population, which is a major medical problem.

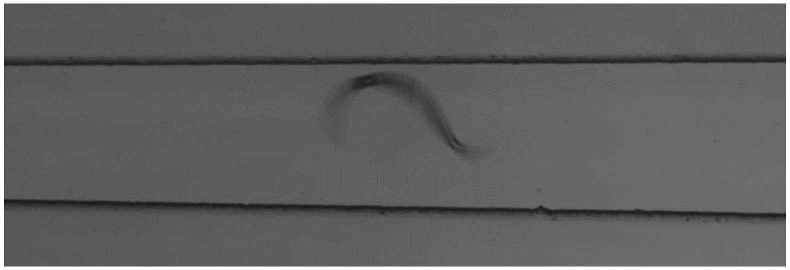
Microfluidics is a useful tool for studying worms, facilitating high throughput assays and enabling the use of motility assays to study the processes of aging, disease progression, drug effectiveness, and more.

The worm trap

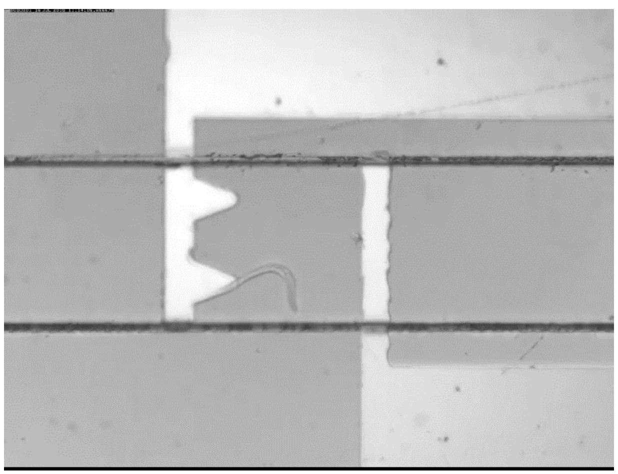
Design Space

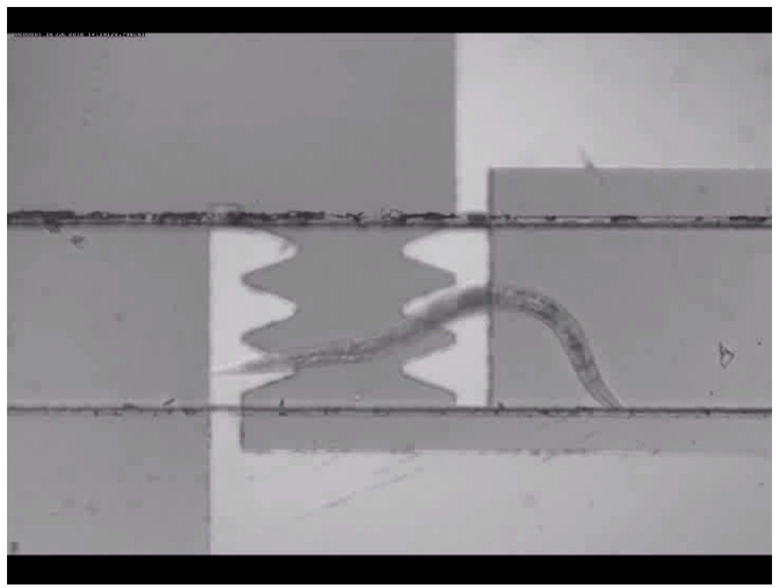


A worm in a drop



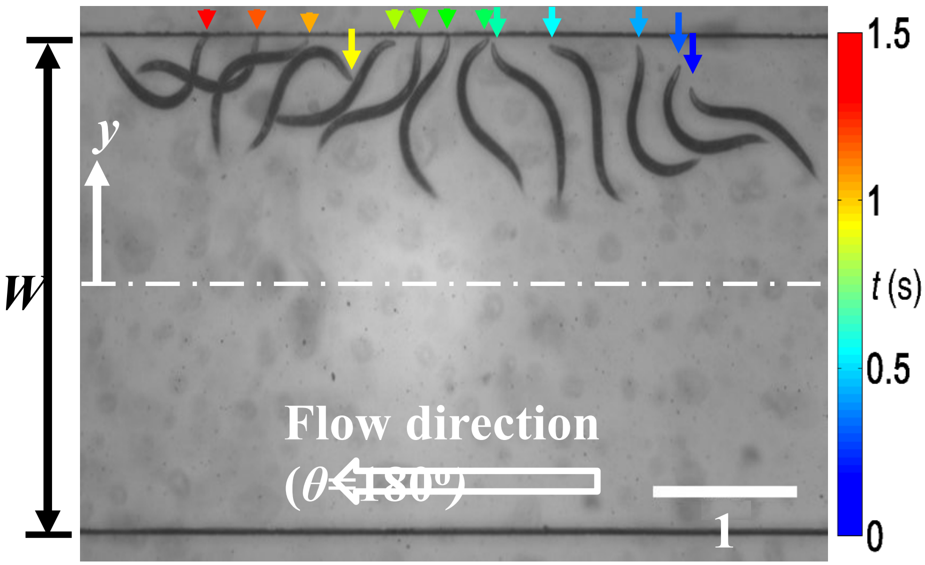
The Infinity Pool Apparatus – the worm swims in one place

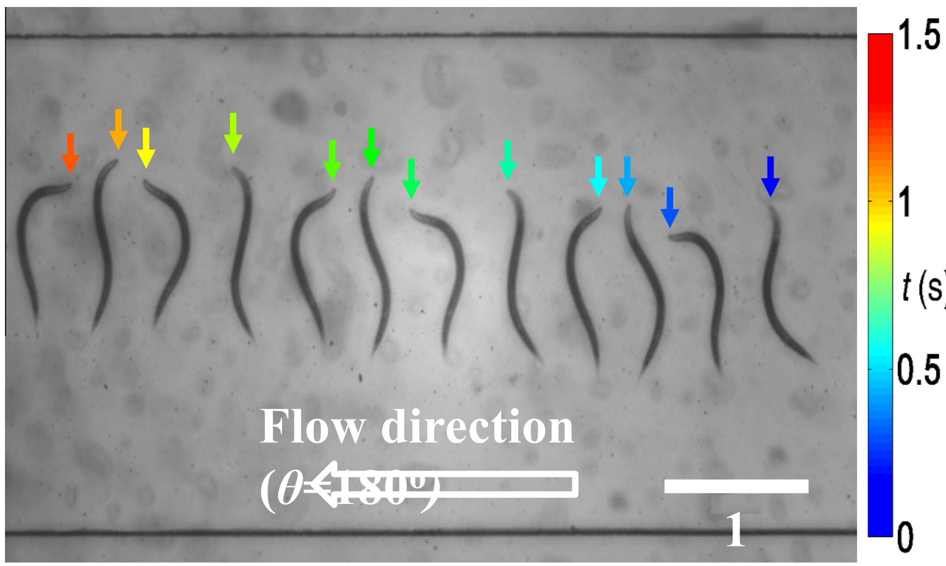


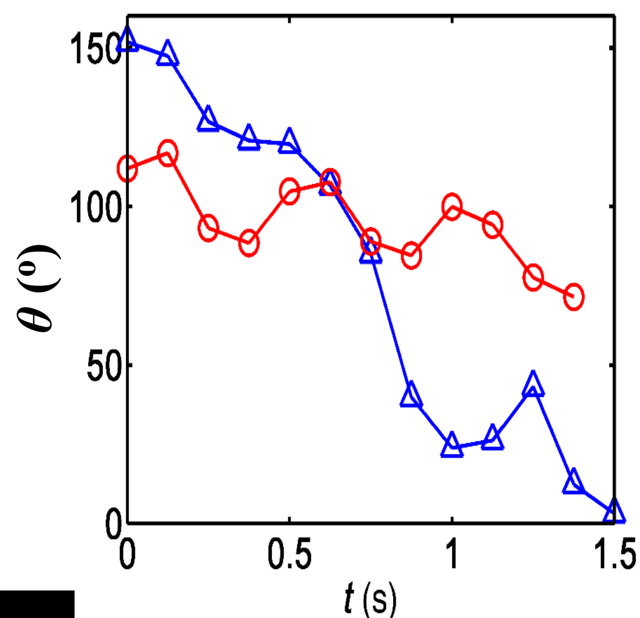


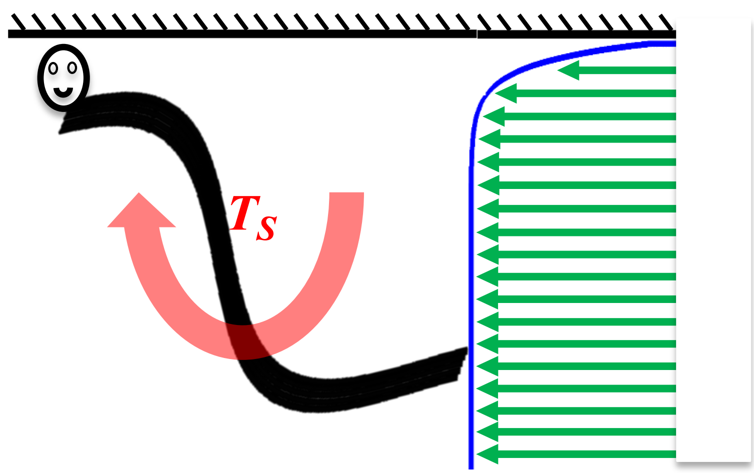
Catch the worm by its tail

Rheotaxis of *C. elegans*: More Pronounced near Surfaces









08:55