Electronic Supplementary Information (ESI) for Zombie Diatoms: Acoustically Powered Diatom Frustule Biotemplated Microswimmers

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1 Growing the Aulacoseira granulata

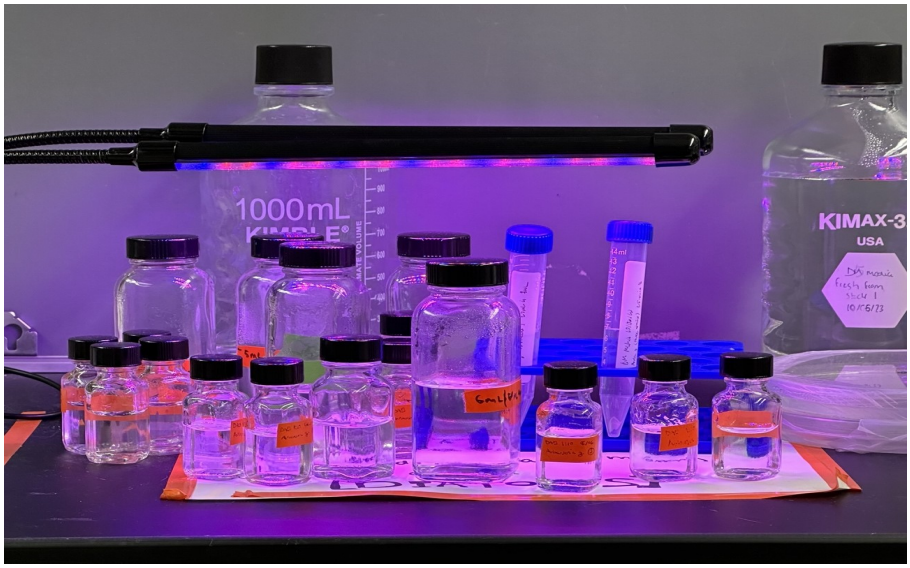


Figure S1 Diatom growth setup for low scale production in a lab that uses cheap commercially available light source and containers.

We cultivated the diatoms by placing approximately 8 parts in volume of the commercial sample into 100 parts of the bulk diatom media (DM) both sourced from Culture Collection of Algae and Protozoa, SAMS Limited, SAM, Dunbeg, OBAN, PA37 1QA, Scotland, United Kingdom) under aquarium lighting set to 12h day/night cycle in 2-week cycles.

2 Characterization of the Anatomy of Our Strain

Anatomy was characterized using scanning electron microscopy, without any coating or processing on copper tape

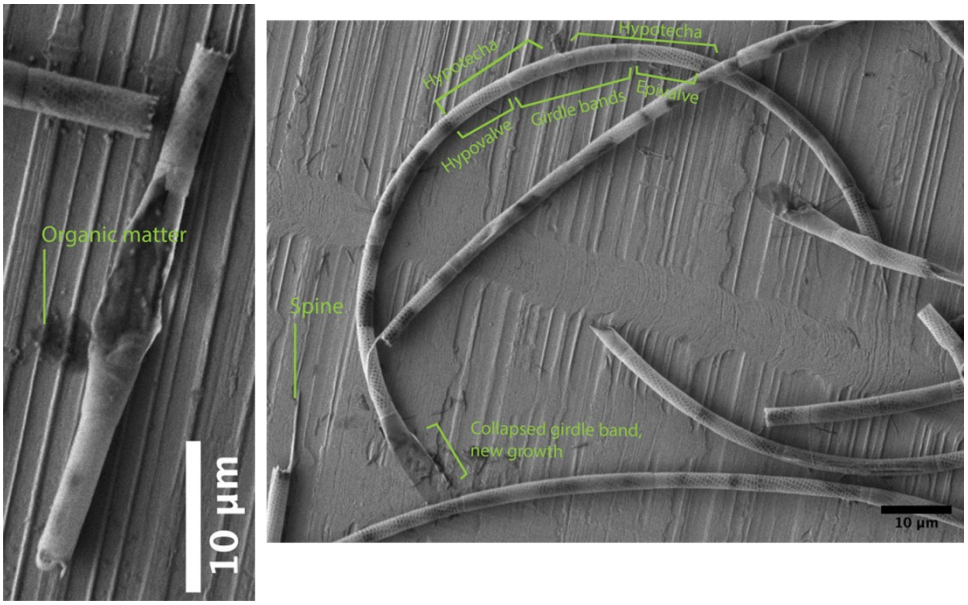


Figure S2 SEM micrograph labeling relevant units of the diatom.

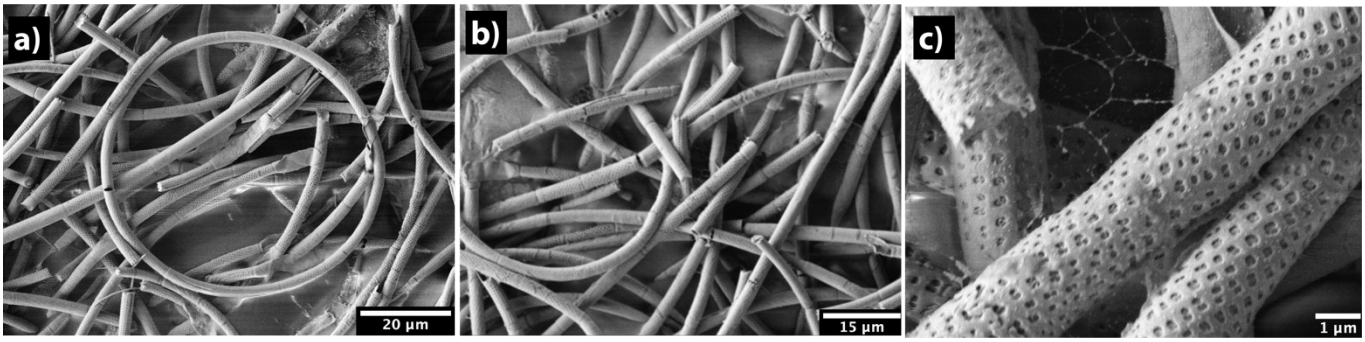
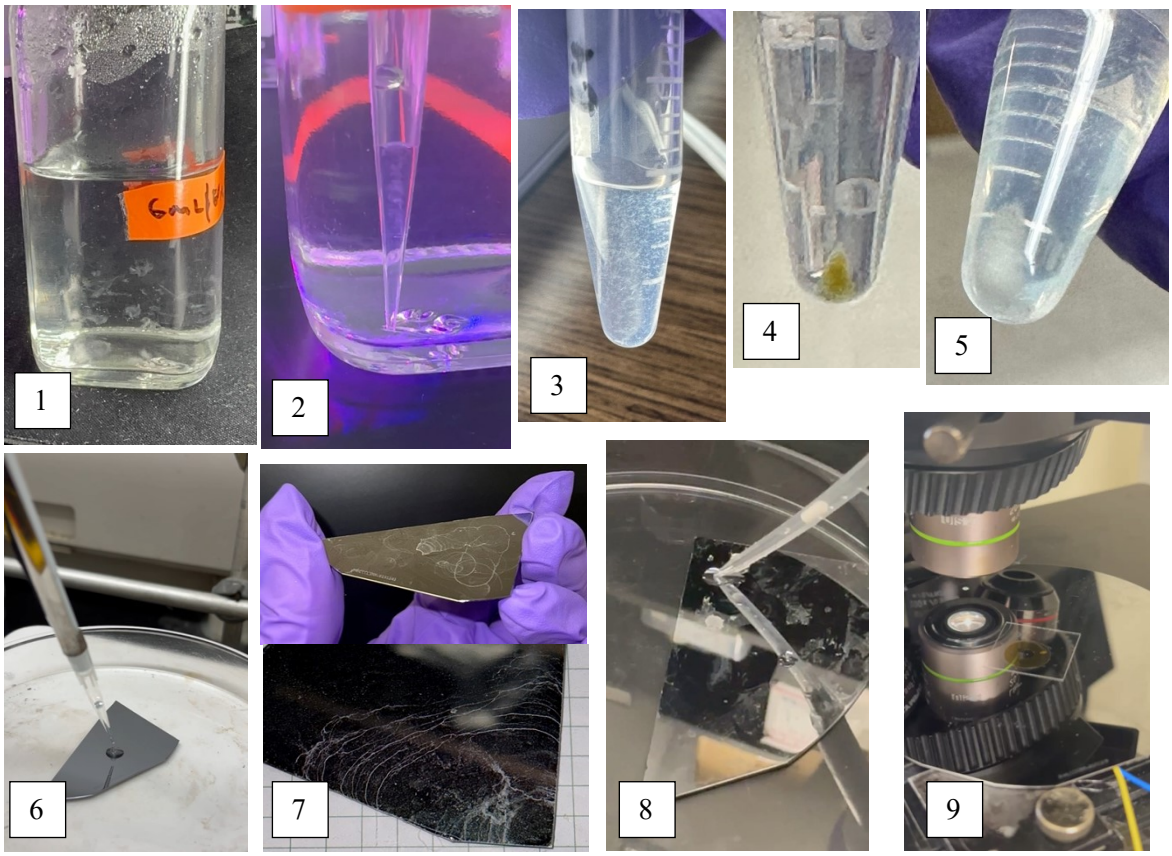


Figure S3 SEM micrographs showcasing the geometries of the unprocessed diatom population: a) large circular filamentous colony b) many intertwined diatoms, c) nanoscale features on the surface of the frustule.

These images allowed us to predict we could separate units of the cylindrical frustules at the links between valves of adjacent frustules, and between valves and girdles within the frustule of a diatom cell.

Figure S4 SEM micrographs of naturally separated diatoms: a) a diatom separated at the ’valve’ end originally connected to another valve via dove-tail-like connections b,c) diatoms separated at the interface-fit, organically and friction held connection between the girdle bands and the solid sections containing the valve.

3 Collecting, Cleaning and Activating the Frustules



Step	Description
1	The diatoms grown appear in colonies that entangle to form a film like sediment at the bottom of the media. The growth can be checked by shaking the container.
2	When it is time to harvest a new batch of frustules, aliquots rich in the film like sediment are collected as suspensions. At this point, the algae might be barely visible.
3	The collected sample is diluted with 1.5x the volume of the sample with 37% hydrogen peroxide and sonicated in an ultrasonic cleaner for a few hours.
4	The resultant suspension is centrifuged to reveal a green sediment. The supernatant is carefully replaced with pure ethanol.
5	The resultant suspension is centrifuged to reveal a white sediment. The ethanol washing can be repeated.
6	After the washing is completed, the supernatant is replaced with ethanol once again, and suspended in solution for a final time with sonication. Then, the suspension is drop-casted on a hot plasma-cleaned silicon wafer.
7	The drop-casting process is likely to result in some coffee-ringing as seen in the image. To avoid this, one can experiment with various rates of heating. The resultant frustule coated substrate is plasma cleaned, dried overnight in a desiccator and vapor coated as highlighted in the detailed description of the procedure in the following section.
8	The hydrophobic frustules are suspended in around 4.5µL of water with 0.1 wt% SDS by expelling the liquid on the substate, scratching it with the tip of the pipette, and repeatedly aspirating and expelling the liquid 4-5 times.
9	Then, the suspension is placed inside the experimental setup, and a glass slide is placed on the top to make the image clear and limit the swimming to a small plane.

Figure S5 Photos of showing the visual state of diatom frustules in each step from harvesting to deployment as microswimmers.

Harvesting and Cleaning the Frustules

Once the off-white to yellow sediments of diatoms became substantially visible in the growth media, we collected small aliquots that were transferred to a centrifuge tube to make up ~1mL of liquid. (Figure S5, Step 2) To this, we added ~1.5 mL of 37% hydrogen peroxide (Step 2-3). This mixture was then sonicated in an ultrasonic cleaning bath for approximately 2 h. Then, the suspension was centrifuged for 15 minutes at 20817 g RCF (Step 4) and the supernatant was replaced with 200 proof ethanol and sonicated for an additional 2 hours. This step was repeated once before replacing the supernatant with fresh ethanol (Step 5). With the suspension containing the clean, isolated cylinders ready, we drop-cast uniform layers of it onto a hot, plasma cleaned silicon wafer (Step 6-7). It was important to obtain uniform coverage to avoid a coffee-ring effect. Experimenting with water content of the final suspension helped to reduce aggregation. We then dried the resulting frustules in a desiccator overnight.

Activating the Frustules with Hydrophobic Silane

In order to make the frustules hydrophobic, we activated their surface by plasma cleaning for 5 minutes. We then placed the silicon wafer containing the frustules off-center on a hotplate set to 80°C, with a thin glass slide at the very center of the hotplate to hold the silane while it is being vaporized. This arrangement created a temperature difference between the silane vapor and the diatoms. To contain the vapor, we placed a 1cm thick crystallizing dish large enough to cover the wafer and the slide. We then placed a drop of 25µL 1H,1H,2H,2H-perfluorooctyltrichlorosilane (97%) on the slide, replaced the cover and waited for 30 minutes. We found that this very hydrophobic silane evaporated easily, and produced uniform layers at low temperatures, but it is possible to use more or less hydrophobic silanes as well. During the vapor coating process, a thin film of silane covers the dish suggesting that the coating process is underway. After 30 minutes, we removed the cover, turned off the heating and allowed the sample to cool to room temperature in place. This step was essential to obtaining a stable silane coating

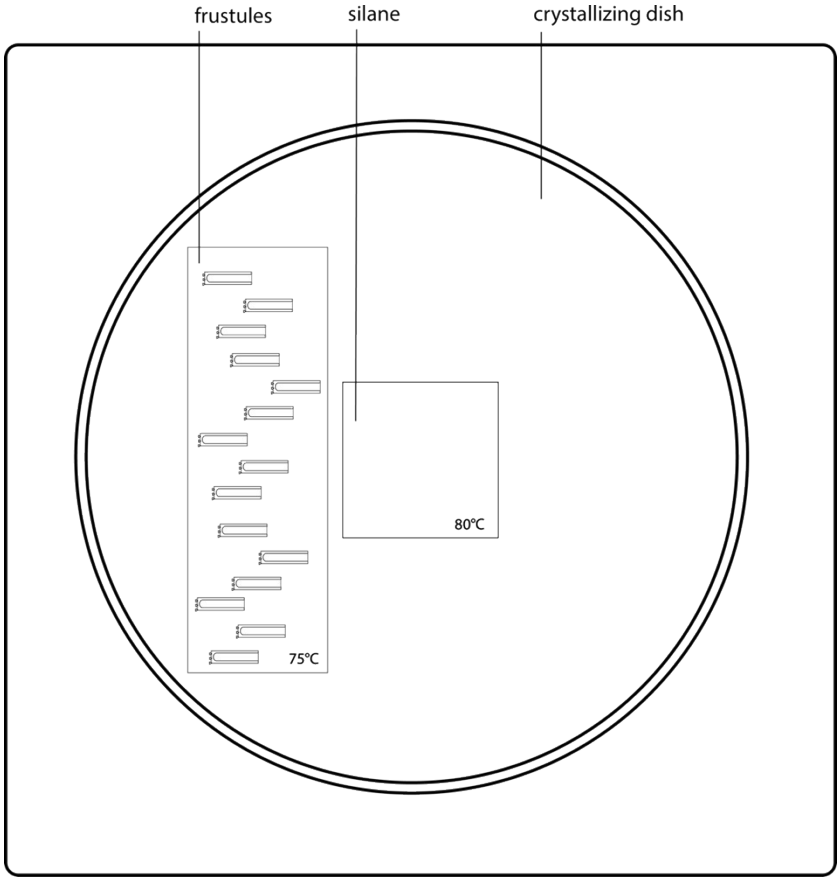


Figure S6 Vapor coating setup on the hot plate. The frustules are placed off center, and the silane is placed at the center with the intension of producing a temperature differential between the silane vapor and the frustules to be coated, which appeared to result in frustules that were more hydrophobic.

Deploying and Observing the Microswimmers

The now hydrophobic frustules are ready to be "scratched-off" from the surface of the silicon wafer (Step 8-9). To do this, 4.5μL of nanopure water with 0.1 wt% sodium dodecyl sulfate (SDS) is dispensed onto a small spot on the substrate covered in the hydrophobic frustules. The tip off the pipette is placed inside the little drop of liquid and the surface is scratched off, while simultaneously aspirating and expelling the liquid.

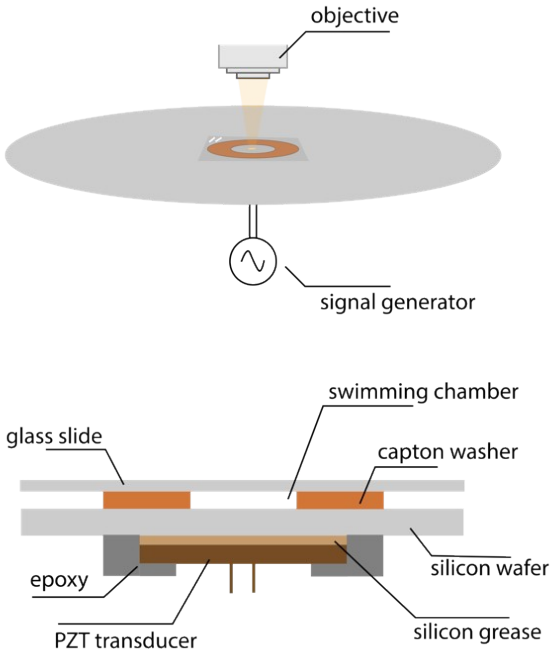


Figure S7 The schematic of the experimental well setup that allows for the frequency and the power of the PZT transducer to be modulated using the attached arbitrary signal generator, while simultaneously observing the swimming behavior under the reflection-mode optical microscope .

4 Bulk characterization of Activity vs. Frequency Through Difference of Consecutive Frames

Any code used is available on request.

Average Mean Square Displacement (MSD) as a function of signal voltage appeared to increase in general.

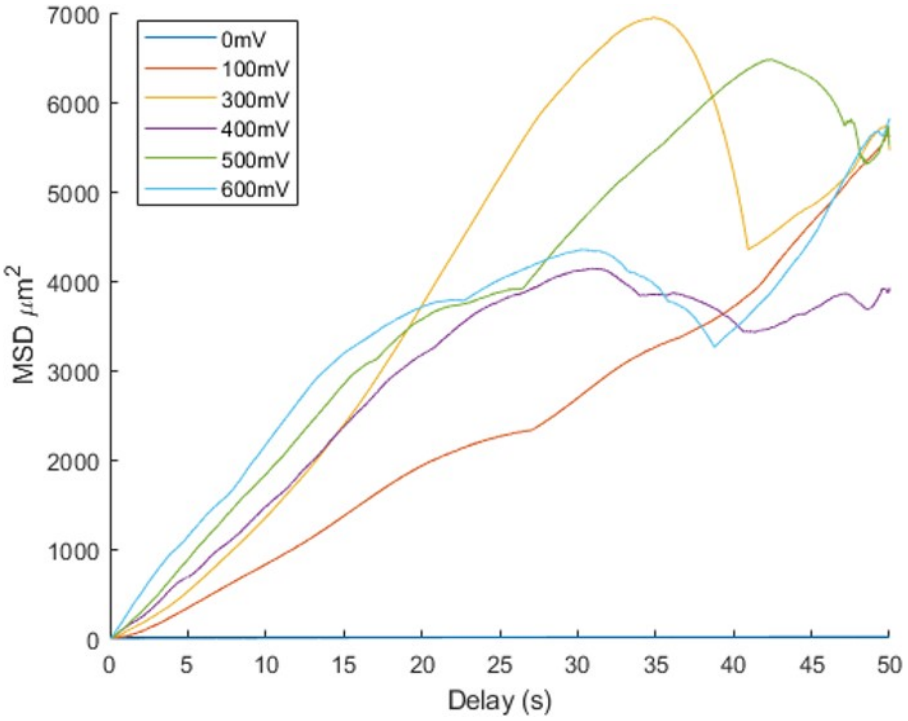


Figure S8 Response of the average MSD vs. Delay of swimmers to signal voltage.

Alpha as a function of signal voltage.

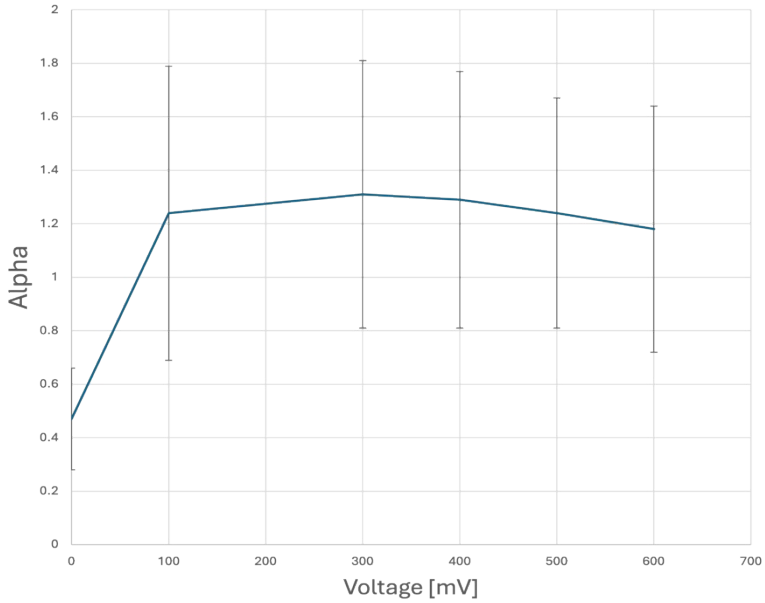


Figure S9 Slopes of the log-log of MSD vs. Delay as a function of signal voltage.

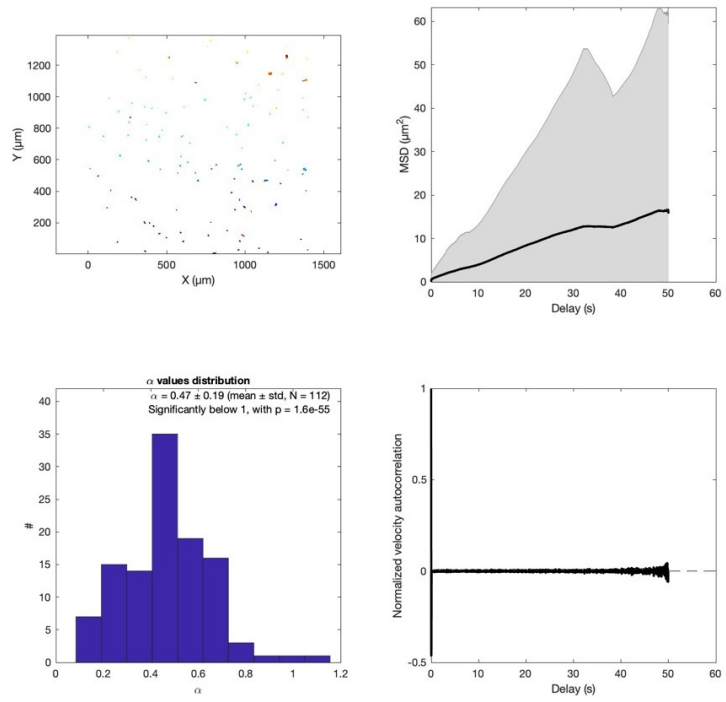


Figure S10 Tracks, average MSD, alpha distribution and normalized velocity autocorrelation plots for diatom based acoustic microswimmers under 0.1% SDS, 0 Vpp transducer voltage conditions. (Control)

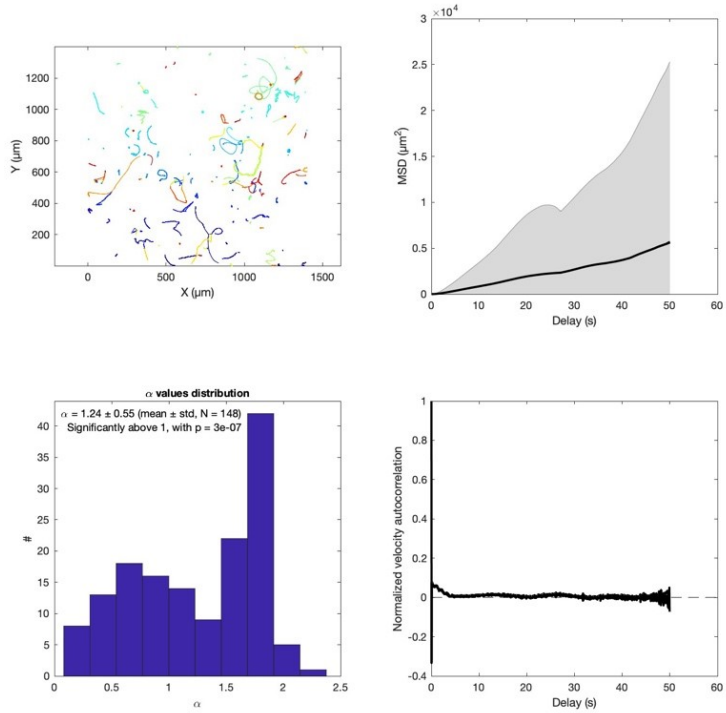


Figure S11 Tracks, average MSD, alpha distribution and normalized velocity autocorrelation plots for diatom based acoustic microswimmers under 0.1% SDS, 0.1 Vpp transducer voltage conditions.

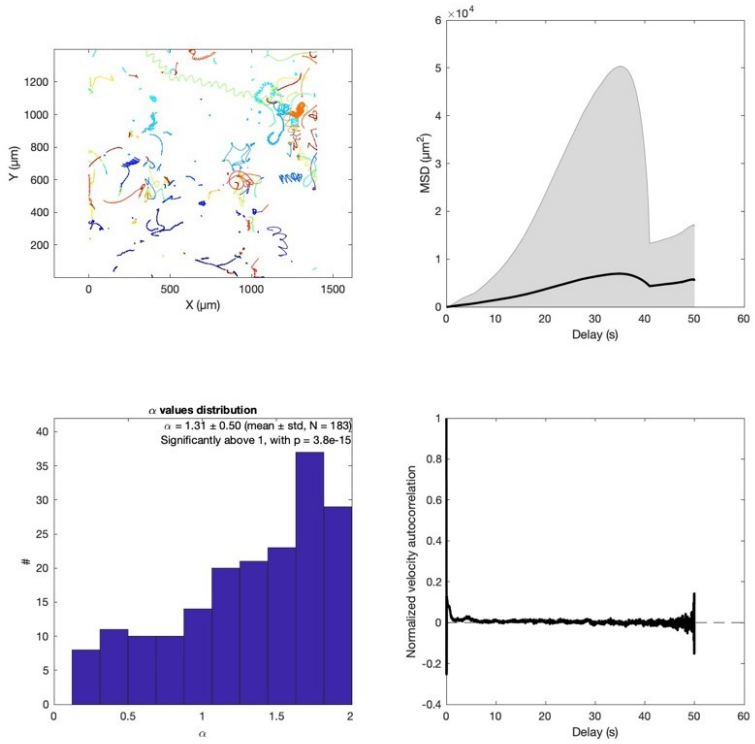


Figure S12 Tracks, average MSD, alpha distribution and normalized velocity autocorrelation plots for diatom based acoustic microswimmers under 0.1% SDS, 0.3 Vpp transducer voltage conditions.

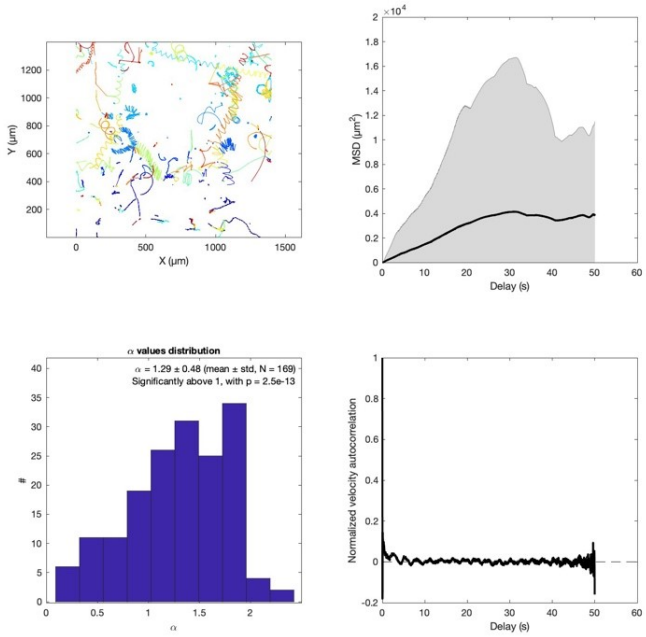


Figure S13 Tracks, average MSD, alpha distribution and normalized velocity autocorrelation plots for diatom based acoustic microswimmers under 0.1% SDS, 0.4 Vpp transducer voltage conditions.

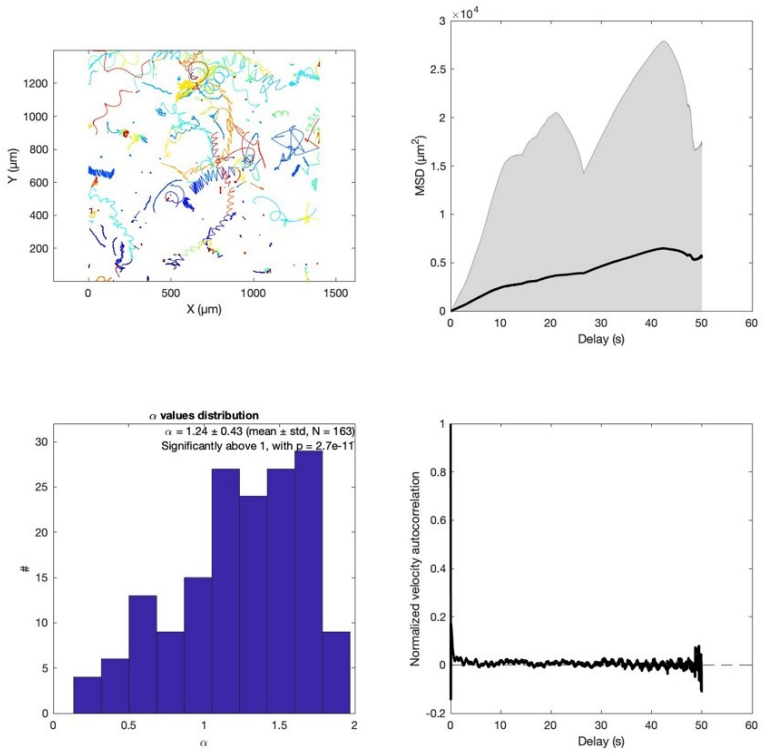


Figure S14 Tracks, average MSD, alpha distribution and normalized velocity autocorrelation plots for diatom based acoustic microswimmers under 0.1% SDS, 0.5 Vpp transducer voltage conditions.

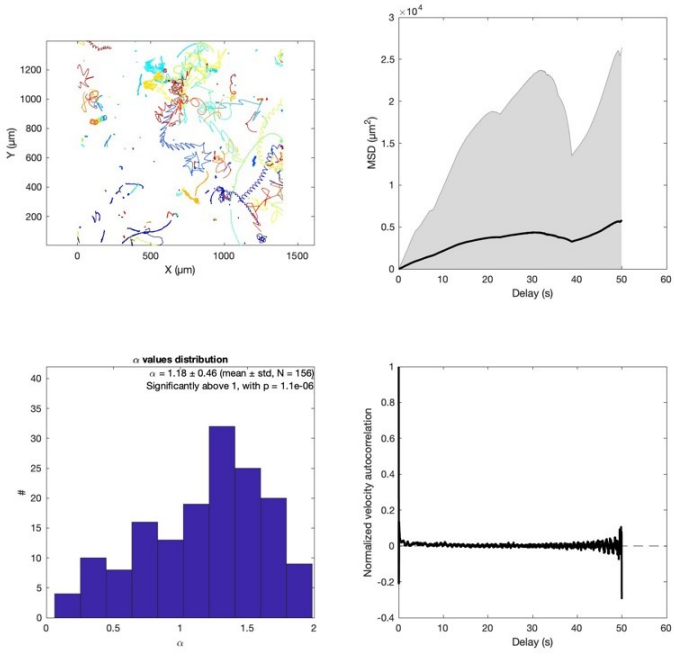


Figure S15 Tracks, average MSD, alpha distribution and normalized velocity autocorrelation plots for diatom based acoustic microswimmers under 0.1% SDS, 0.6 Vpp transducer voltage conditions.

5 Additional Notes & Video ESI Descriptions

Code available upon request.

Video ESI associated contains:

- VIDEO 1: Trajectories with Varying Voltage

The transducer was set to the resonance frequency of 1.04MHz which was determined to be the frequency at which the largest response from the swimmers was observed in the experimental setup. The video contains trajectories of the swimmers at control (0 mVpp), 300 mVpp and 600 mVpp applied to the transducer. This qualitatively demonstrates increased motion in the presence of the appropriate acoustic field, and the lack of it when there is no sound energy present.

- VIDEO 2: Complex Trajectories Close-Up

Raw footage of frustule-based swimmers at 1.04 MHz and 0.5 Vpp sinusoidal signal provided to the transducer. Note that the different ways in which the silica-based frustule, water and air interfaces refract light make the bubbles that are trapped inside the cylindrical cavities visible. It appears that some frustules are still attached as two “cap” shapes back-to-back and most are singular. There appears to be various ranges of speeds, levels of interaction with the silicon wafer and the glass slide. Most appear to be floating by default, as they move in a pseudo-planar fashion scraping on the glass slide at the top.

- VIDEO 3: Self Assembly

Close-up video showcasing the complicated ways in which the frustule-based swimmers can move and transiently interact. For example, at 1-4 s of the video, the swimmer at the bottom left of the frame is observed swimming in a spiral, when it encounters and couples with the bubble of another frustule and assembles into filament structure at 5 s, followed by its eventual autonomous separation at 9 s.