Supporting information

Microswimmers under the spotlight: interplay between agents with different levels of activity

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1 List of movies provided as Supporting Information

1. Movie 1: Templated inverse assembly. The central square region is illuminated, and the light is switched OFF everywhere else.
2. Movie 2: Templated assembly. Light is OFF in the central square region and ON everywhere else.
3. Movie 3: Templated assembly of large clusters. Light is OFF in the central region with a width an order of magnitude larger than the persistence length of the active fluid.
4. Movie 4: Assembly process when a light pattern with a checkerboard motif is applied to the system.

2 Additional details on sample cell preparation

As discussed in the manuscript, with the particles in close contact with a sample cell's surface, there is a potential for strong interaction with the substrate and steps must be taken to prevent any undesirable interaction. To wash the surface of a substrate, we adopt the following protocol:

1. Wash with a cleaning solution (Hellmanex^{TM}, Sigma-Aldrich),
2. Rinse thoroughly in deionized water (Milli-Q Advantage A10, Millipore),
3. Plasma clean (Plasma-Prep II, SPI Supplies) for at least 20 min.

The above protocol removes unwanted debris from the surface, reduces the attraction between the swimmer and the substrate, and improves, though perhaps marginally, the imaging quality. The swimmer's hematite surface continually catalyzes an oxygen-producing reaction when illuminated with a sufficiently high-frequency light source. When the amount of dissolved oxygen exceeds the solubility in water it will nucleate into bubbles that rise to the top of the sample. For this reason, how a sample cell handles the accumulation of bubbles will be critical for evaluating its utility during long experiments. Below, we will outline several cell designs, while evaluating the reasons for and against the use of each cell design.

1. Borosilicate capillary tubing: The solution is loaded into a glass capillary tube by submerging one of the ends of the tube into the sample solution and allowing it to fill via capillary forces. The tubes are

sealed at either end with cured optical adhesive (NOA68, Norland). To avoid premature nucleation of oxygen, capillary tubes are covered with an aluminum foil such that only the ends are exposed when being cured under UV radiation. Here we present the two geometries of capillary tubing used along with their advantages and disadvantages:

(a) A rectangular capillary tube with inner dimension $0.1mm \times 2.0mm$ (VitroCom # 5012). With a particle sedimentation velocity of $v_{sediment} \sim 1.4\mu m/s$, the sample is ready for experimentation in just over 1 min. These tubes are typically used for experiments done at low particle density or within a relatively short amount of time for high particle density. Experimentation with these tubes cannot be carried out for very long because very large bubbles spanning the height of the sample cell will impede the motion of individual swimmers.

(b) A square capillary tube with inner dimension $1.0mm \times 1.0mm$ (VitroCom # 8100). A sample cell with a larger height will require a longer time for all particles to sediment to the bottom of the sample cell, so the sample is ready for experimentation in just over 10 min. These tubes are typically used for experiments done at high particle density because the likelihood of an experiment running long enough for an oxygen bubble to span the entire height is extremely low. The downside to using these capillary tubes is that large bubbles translating across the top of the tube will distort the image of the microscopic scene by shifting contrast, acting as a lens, and by casting a shadow. Though this bubble does not impede the motion of the swimmers, filtering the shadow from the scene takes time in the post-processing stage.

2. Photolithography patterned wells: A sample cell of wells patterned through standard photolithographic techniques. SU-8 wells with various in-plane geometries and heights ranging from $20\mu m - 100\mu m$ are created on glass coverslips ($45 \times 50 - 1.5$, ThermoFisher Scientific). Outer walls are constructed from laser-cut acrylic (height 6.35 mm) that is affixed to the surface of the SU-8 with optical adhesive (NOA68, Norland). Once the sample solution is loaded into the chamber, it is covered with a clean glass coverslip that is held in place with Scotch tape. The significant height and width of the outer walls allow for long-term experiments at high density and the well allows for experimentation in confinement. The oxygen bubbles will not affect the imaging for these experiments, but the shadows from the edge of the wells will require image post-processing. This entire process of creating a sample cell is time intensive and there are a few points of failure that cannot be discovered until well into experimentation. Some examples include:

   (a) There may be residual SU-8 left on the surface of the glass. With a refractive index close to glass at small heights, this can go unnoticed until the measurement of particle dynamics is in the data analysis step.

   (b) The adhesion of SU-8 on glass is not permanent and it will eventually peel off, as the sample cell undergoes multiple cleanings in preparation for experimentation.

   (c) The adhesion of acrylic on SU-8 on glass is not permanent and it will eventually peel off, especially as the sample cell undergoes multiple cleanings in preparation for experimentation. This deformation in the wells can cause unwanted flows which can be subtle enough to not be noticed by the eye during experimentation.

3. Glass wells: A sample cell created with a commercially purchased glass cylinder. As with the sample cell built around the photolithographic wells, the height allows for long-term experimentation without the disadvantage arising from oxygen bubbles casting shadows on an image. A cloning cylinder (#09-552-1, Pyrex) with a diameter of 8 mm and height of 7.87 mm adheres to a glass coverslip with the optical adhesive (NOA68, Norland). Once the sample solution is loaded into the chamber, it is covered

with a clean, thin slab of cured PDMS. The PDMS lid prevents debris from entering the sample cell while allowing excess oxygen to diffuse through its pores. The disadvantage to using a cell with such a large height is that it will take around 1:5 h for the colloidal particles to sediment.