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Microchamber slide design for cell confinement during imaging

Cells can be highly motile, moving in and out of a microscope's field of view. Understanding complex life cycles is difficult without continuous observation. To overcome this challenge, we've developed a 3D-printed microchamber device to confine cells for long-term visualization.

Contributors (A-Z)

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Purpose

We want to understand how protists survive desiccation when ephemeral bodies of water become dry. One such protist, *Colpoda steinii,* has a complex life cycle with a motile state and at least two distinct static states. The motility of cells presents a challenge to observation because cells will swim out of the field of view. We have developed microchambers to confine cells during imaging experiments. The

confinement allows us to track and phenotype cells across distinct cellular states, over long periods of time.

- This pub is part of the **project**, "Dissecting mechanisms of environmental adaptation in protists." Visit the project narrative for more background and context.
- **3D printing files** are available at the NIH 3D Print Exchange.
- All associated **code** is available in this GitHub repository.
- **Imaging datasets** associated with this pub are available in these Zenodo repos: *C. steinii* cyst rotation, early encystment, in a 384-well plate or microwells, and reanimation; and *Tetrahymena rostrata* swimming.

The strategy

Cells can have complex life cycles, with transitions between active and dormant states **[1][2]**. Observing cells across the life cycle is essential for understanding their complex behaviors. Also, observing cell state changes lets us phenotype organisms after experimental perturbation.

Colpoda steinii is a protist that thrives in ephemeral bodies of fresh water, such as dew that forms on plants, or creeks that dry up. In its active state, when in the presence of a nutrient source (e.g. bacteria), *Colpoda* is swimming or dividing. Upon starvation, cells go dormant, forming a resting cyst that can survive desiccation. We want to understand the molecular mechanisms that underlie encystment and excystment, and it's important that we're able to observe *Colpoda* life cycle changes and other phenotypes under the microscope.

Problem

During microscopy experiments, active *Colpoda* swim out of the field of view. The free dispersal of cells outside the field of view precludes continuous visualization across changes throughout a life cycle. Another problem is that *Colpoda* will form cysts on the walls of the chamber, where the camera cannot detect them.

Solution

To overcome the challenges of “invisible” or “disappearing” cells, we have developed microchambers to observe and record *Colpoda steinii* across transitions in its life cycle.

The resource

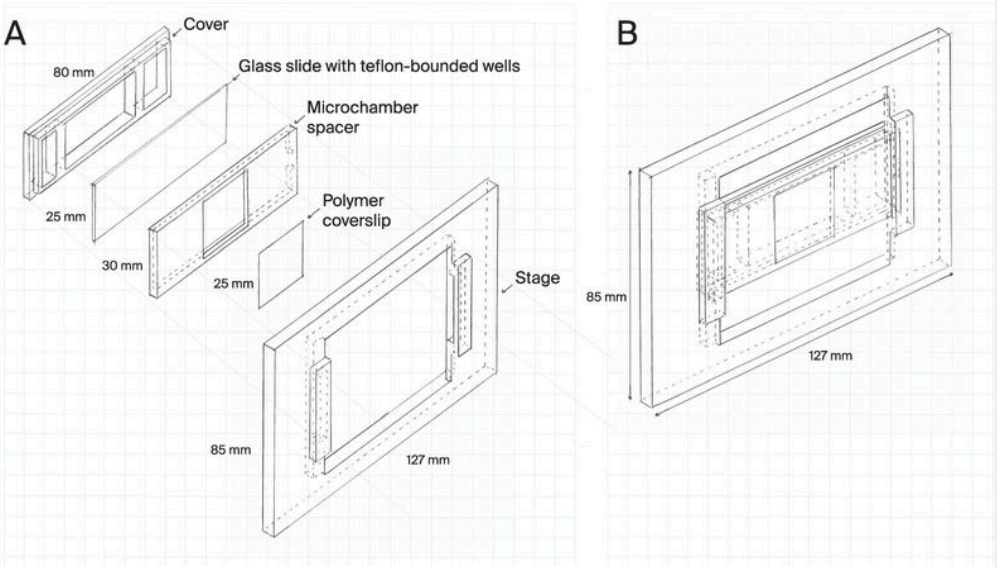


Figure 1

Diagram of the microchamber slide components.

(A) Expanded view of all components.

(B) Fully assembled apparatus.

The apparatus as a whole consists of several parts (Figure 1), some custom-3D-printed and others commercially available. A 3D-printed spacer defines the Z dimension of the microchamber. A teflon coating on the glass slide defines the boundaries of the microchambers in the X and Y dimensions. A gas-permeable coverslip caps the microchambers and enables oxygen exchange to maintain the health of the cells in the chamber. A 3D-printed cover helps hold the slide next to the spacer and coverslip. Finally, a 3D-printed stage holds the microchambers on an inverted microscope.

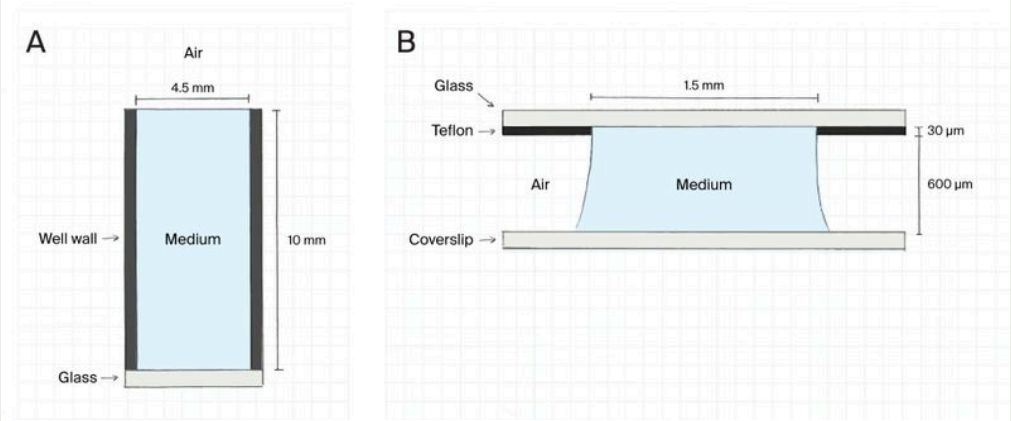


Figure 2

Side views of a well from a 384-well plate (A) and a microchamber (B).

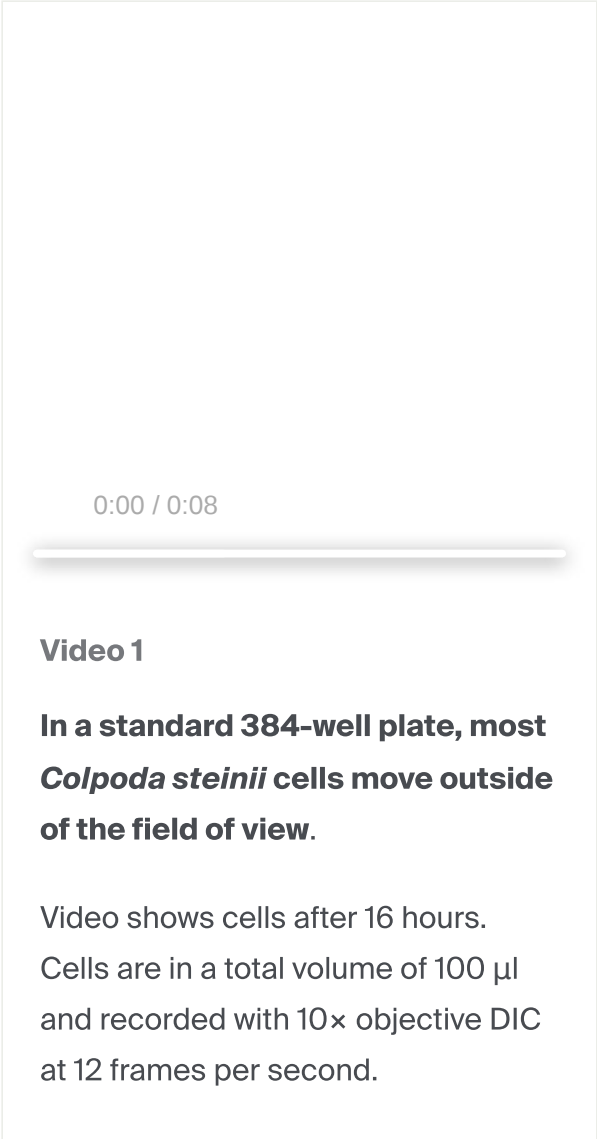
Note that the scale is distinct between panels.

The “walls” of the microchambers are the air-liquid interface (Figure 2). *Colpoda steinii* cells encyst on a solid surface, so resting cysts remain visible on the gas-permeable coverslip or on the glass slide. The volume of the microchamber can be altered by changing the diameter of the well on the teflon-coated slide or by changing the height of the 3D-printed spacer according to specific experimental needs. The coverslip should be glued to the spacer prior to using the apparatus for live imaging. Rubber bands hold the assembly together.

For more information on the components and assembly, including catalog numbers, jump to the “Materials and methods” section.

PRINT IT: All STL and printing files are available in the NIH 3D Print Exchange.

The microchamber apparatus is effective at confining cells for imaging over several hours

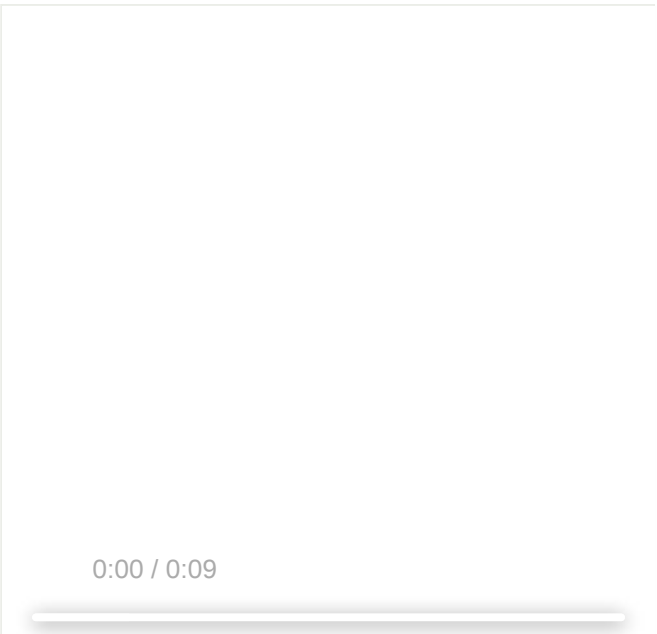


Video 1

In a standard 384-well plate, most *Colpoda steinii* cells move outside of the field of view.

Video shows cells after 16 hours. Cells are in a total volume of 100 µl and recorded with 10× objective DIC at 12 frames per second.

To test whether our microchamber design could effectively confine cells, we placed *Colpoda steinii* cells in either a well of a 384-well plate (Movie 1) or in a microchamber (Movie 2), periodically collected DIC images over the course of 16 hours, and then counted cells in the images.



Video 2

Within microchambers, many more cells stay in focus.

Video shows cells after 16 hours. Cells are in a total volume of 1.6 µl. Microchamber diameter is 1.5 mm and depth is 400 µm. Recorded with 10× objective DIC at 12 frames per second.

NOTE: This experiment used an early prototype that didn't have a cover to help secure all of the pieces together. The volume is larger than expected, because the pieces were not in apposition. For comparison, recent prototypes with a cover and rubber bands have a volume of 1 µl with a 600 µm spacer.

The microchambers confined the population of *Colpoda* such that 30–40% of the cells were in focus in the field of view (Figure 3). In contrast, only ~2% of the total cells were in a similar-sized field of view in the well of the 384-well plate. These results suggest that the microchambers increase the chance of a cell appearing in the field of view at any given time point.

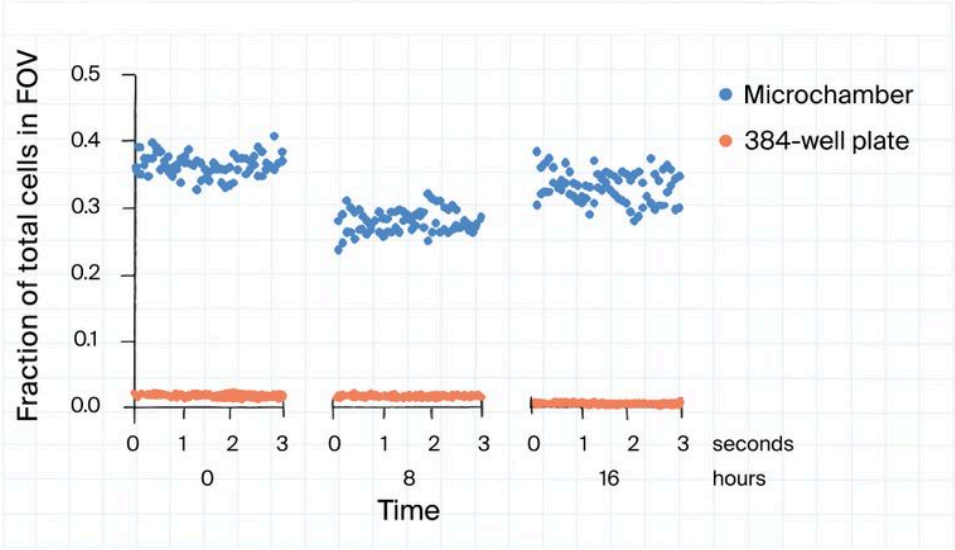


Figure 3

Substantially more *Colpoda steinii* cells are in focus in the microscope field of view when confined in a microchamber.

Cells imaged in a 384-well plate are indicated in orange, and those confined in a microchamber are displayed in blue.

In both chambers, we observed *Colpoda steinii* cells swimming throughout the 16 hours of data collection, suggesting that the cells stay alive and healthy while confined. However, in previous failed prototypes using a glass coverslip instead of the gas-permeable coverslip, cells stopped swimming, presumably due to a depletion of oxygen in the chamber. After observing cell death in the microchambers when using glass coverslips, we wondered whether confinement in the microchambers with a gas-permeable coverslip perturbed the physiological behavior of the organism. We chose to test cell perturbation in the microchamber using unicellular algae with a known swimming behavior — *Chlamydomonas reinhardtii* **[3]**.

Confining cells in microchambers does not perturb or restrict cell behavior

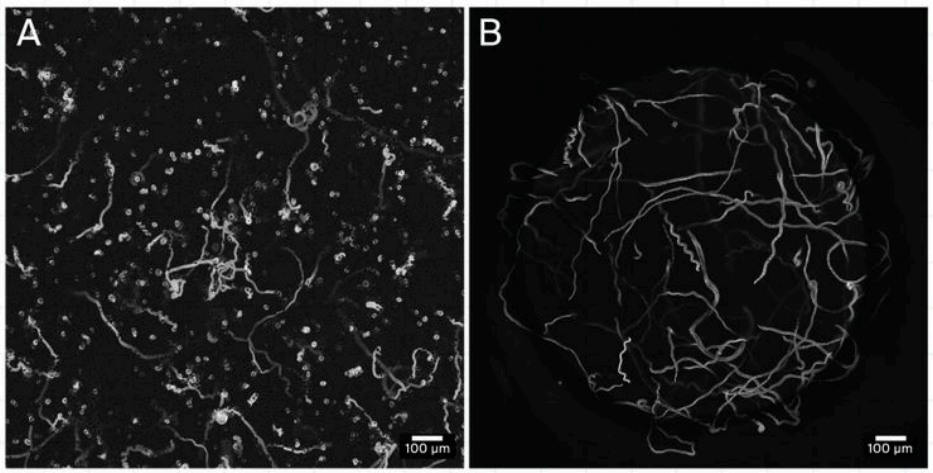


Figure 4

***Chlamydomonas reinhardtii* cells maintain swimming behavior when confined in microchambers for 24 hours**.

Images show tracks of moving cells over the course of 10 seconds of video capture at 40 frames per second. We performed frame subtraction (frame 12 minus frame 1, etc.) for each frame in the video to isolate signals from moving cells, and performed a maximum intensity projection on the resulting image stack.

(A) Cell tracks in a well of a 384-well plate after 24 hours.

(B) Cell tracks in a microchamber after 24 hours.

We placed *Chlamydomonas* cells in either a well of a 384-well plate or in microchambers on the same day, taken from the same starting culture. We incubated both types of vessels at room temperature in a humid environment and imaged cells after 24 hours of confinement. Imaging parameters on the microscope were constant between the two samples. In both vessels, we observed cells swimming at the 24-hour time point (Figure 4). In the microchambers, many of the swimming tracks of the cells were long, and different swimming patterns were clearly visible. Swim paths with a

spiral pattern are clearly distinguished from swim paths with a straight trajectory (Figure 4, B).

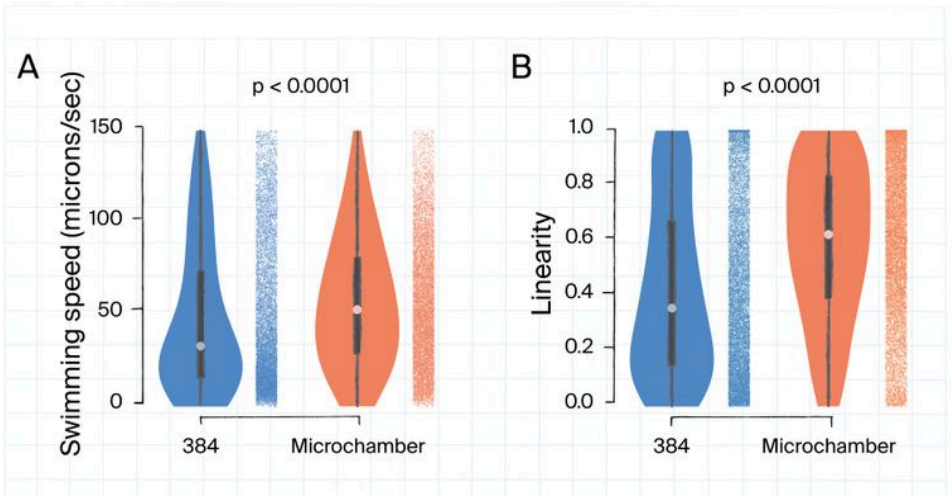


Figure 5

***Chlamydomonas* cells display enhanced swim speed and swim direction linearity in microchambers**.

(A) Frame-to-frame displacement of cells.

(B) Linearity of swim trajectories.

Both measures are presented as violin plots with the associated data points displayed next to each violin (Kruskal-Wallis p-value).

Cells in 384-well plates swam at an average speed of 47.32 µm/second while those in microchambers swam at an average speed of 56.99 µm/second (Figure 5, A). These measurements are similar to those reported in the literature **[3]**, but we observed significantly faster swim speeds in the microchambers. The speeds could appear faster in the microchambers, because there is less distance to travel in the Z dimension in the microchamber. For example, if a cell is swimming straight up at the camera, it will make a shorter trace in the same amount of time. Alternatively, the observed faster swim speeds could be a response to more even illumination in the microchamber compared to the 384-well plate. The dark walls create uneven illumination in the well. This is visible in Movie 1, where the sample is less illuminated in the bottom-left portion of the image compared to the top right. Also, the medium absorbs a fraction of the light through the vessel. The observed faster swim speeds

could also be a response to enhanced illumination in the short water column (600 µm) of the microchamber compared to the long water column (10 mm) of the 384-well plate.

Furthermore, the swimming trajectories of cells in the microchambers were significantly more linear (Figure 5, B). This is partially due to the smaller fraction of cells resting on the microchamber coverslip, compared to the 384-well plate, which were rotating in tight circles.

Overall, these data suggest that the microchambers enable robust observation of unperturbed cell swimming behavior, even after extended periods of cell confinement.

Video 3

In a vessel with a long water column immotile cells sediment to the coverglass and obscure motile cells.

Video shows *Chlamydomonas reinhardtii* at a 24 h time point. Cells are in a total volume of 100 µl in a 384-well plate and recorded with 10× objective DIC at 40 frames per second. The water column has a height of ~10 mm.

In the course of the experiment, we observed an unexpected benefit of imaging in a microchamber with a relatively short water column. In populations of *Chlamydomonas,* a fraction of the cells are not swimming — these sediment to the surface of the vessel. At a fixed concentration of cells, vessels with a longer water column will have more nonmotile cells on the bottom surface. This is exactly what we observe when comparing the 384-well vessel and the microchamber. The water column in the 384-well plate is ~10 mm (100 µl total), whereas the water column in the microchamber is 600 µm (1 µl total). There are clearly more nonmotile cells in the 384-well plate (Movie 3) compared to the microchamber (Movie 4).

0:00 / 0:09

Video 4

In a vessel with a short water column there are fewer immotile cells that sediment to the coverslip.

Video shows *Chlamydomonas reinhardtii* at a 24 h time point. Cells are in a total volume of 1 µl in a microchamber and recorded with 10× objective DIC at 40 frames per second. The water column has a height of 600 µm.

This means that imaging in a microchamber allows us to see a spectrum of cellular behaviors at a single plane. If we were to image cells with complex life cycles in a well of a 384-well plate, we'd likely have to image both close to the coverslip to see nonmotile cells (like cysts) and deeper in the well to see the motile cells. These results thereby inform vessel choice when studying cellular behaviors in imaging experiments.

Materials and methods

We want others to be able to use this design as well, so we're sharing more details here. We'd love to hear if you try this, and please leave a comment if you need any additional information.

Apparatus components

We designed 3D-printed parts using Autodesk Fusion 360 software (2.0.13619) and printed them with a Formlabs Form 2 printer (Firmware version rc-1.19.25-1085) using grey (FLGPGR04), white (FLGPWH03), or black (FLGPBK03) resin from Formlabs. We've successfully printed parts with a 25 µm or 50 µm layer thickness.

NOTES: Use of different resins resulted in parts of slightly different sizes. When needed, we sand parts down to appropriate size to fit together. You can sterilize all 3D-printed parts and the coverslip with 70% ethanol, even after assembly.

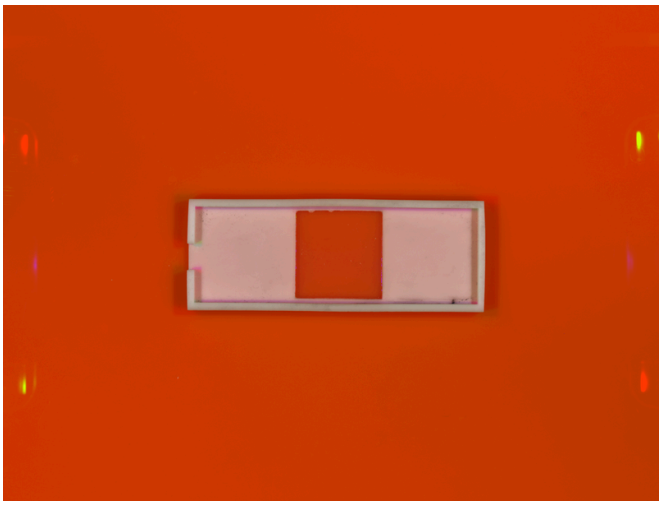
> **PRINT IT**: All STL and printing files are available in the NIH 3D Print Exchange.

Individual components of the microchamber apparatus:

1. A Teflon-coated glass slide (25 mm × 75 mm) with holes (1.5 mm diameter) in the Teflon that define the X and Y dimensions of the microchambers.

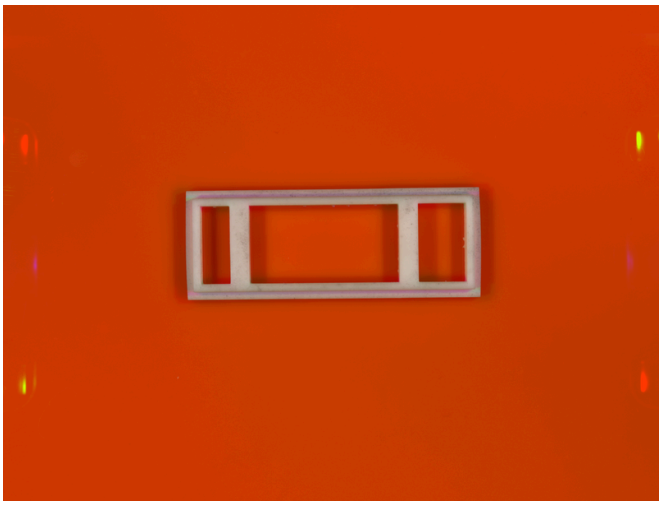
Obtained from Tekdon (192 well / 1.5 mm Slide ID: 192-150).

1. A 3D-printed spacer that defines the Z dimension of the microchambers.



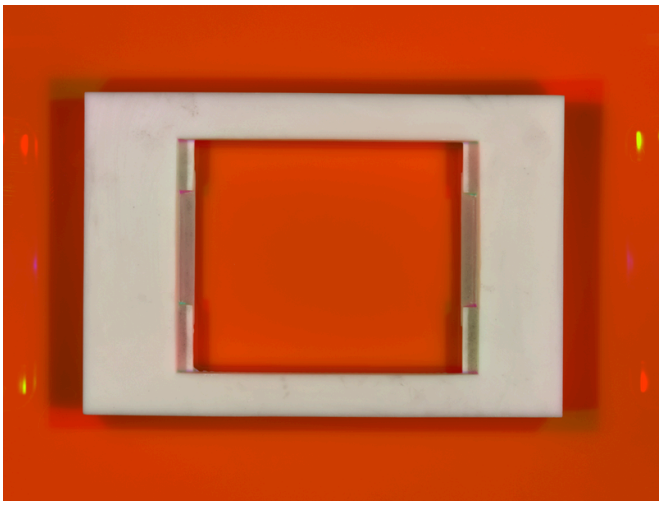
Microchamber spacer, 3D-printed with white resin.

1. Gas-permeable coverslip, 25 mm × 75 mm. The coverslip caps the microchambers and enables oxygen exchange to maintain the health of the cells in the chamber.
   Obtained from Ibidi (catalog #10814).
2. 3D-printed cover helps hold the slide next to the spacer and coverslip.



Cover, 3D-printed in white resin.

1. A 3D-printed stage that supports the microchambers on an inverted microscope.



Stage, 3D-printed with white resin.

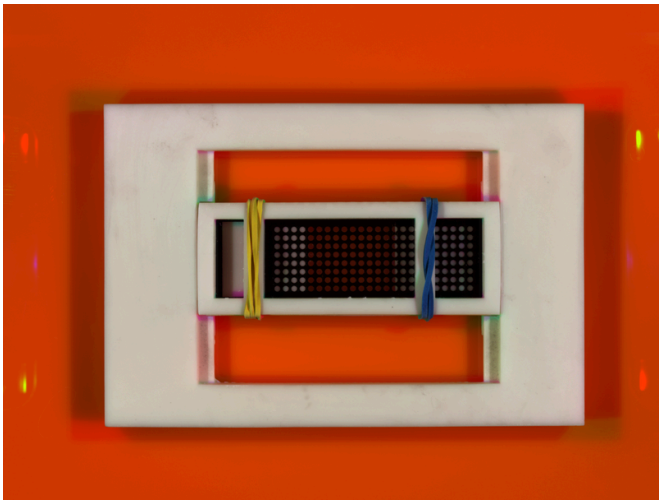
1. Original Gorilla glue adheres the coverslip to the spacer.
2. Rubber bands secure the cover to the spacer and maintain the height of the microchambers.
3. 25 mm × 25 mm square 3D-printed pad cushions the coverslip while clamped to the spacer as the adhesive sets.
4. Binder clip, or some other gripping tool, clamps the spacer to the coverslip as the adhesive sets.

PRINT IT: All STL and printing files are available in the NIH 3D Print Exchange.

Preparation of the apparatus

1. Use scissors to cut polymer coverslip to 25 mm × 25 mm.
2. Apply glue to the edges of the internal hole of the spacer.
3. Layer Kimwipes and parts in the following order: glass slide-Kimwipe-spacer-coverslip-kimwipe-25 mm square pad.
4. Clamp for five minutes.
5. Disassemble, replace the Kimwipes with fresh ones, reassemble, and clamp.
6. Clamp overnight to cure.

7. Disassemble the clamped pieces.



Assembling the apparatus.

We place cells in 1 µl of medium into each well on the teflon-coated slide in a humidified chamber. We then place the slide on the 600-µm spacer. The liquid will make contact with the coverslip. Then we place the cover on top of the slide and secure the sandwiched parts with rubber bands. Finally, we place the microchamber assembly on the stage as shown.

Cell culture

We cultured *Colpoda steinii* (American Type Culture Collection 30920) by weekly reanimation of resting cysts with filtered (0.2 µm) hay medium supplemented with an overnight inoculum of *Klebsiella aerogenes* (American Type Culture Collection 13048). On the day after reanimation, we inoculated culture supernatant containing swimming *Colpoda* into a vessel containing fresh hay medium and bacteria. We incubated cultures in a humid chamber at 25 °C.

We cultured *Chlamydomonas reinhardtii* (CC-125, *Chlamydomonas* Resource Center) in TAP medium (UTEX Culture Collection of Algae) at room temperature (~22 °C) with a 12 h light-dark cycle on a rotating culture wheel.

Microscopy

We performed Imaging on a Nikon Ti2-E & Yokogawa CSU W1-SoRa microscope. The microscope was equipped with an ORCA-Fusion BT digital C-MOS camera. We used a 10× 0.45 Plan Apo Air objective for differential interference contrast (DIC) imaging.

Data analysis

For swim tracking and also for swim speed and swim linearity analysis, we performed image subtraction (frame 12 minus frame 1, etc.) using a Fiji **[4]** macro. Image subtraction isolates the signal that is changing from frame to frame, such as swimming cells. We performed a maximum intensity projection on the frame-subtracted movies to observe swim tracks. We used Cellprofiler **[5]** to identify cells in images, measure frame-to-frame displacement of objects, and measure the linearity of swimming trajectory. We performed statistical analysis of swim speed and linearity using R **[6]**. We compared these measures statistically using Kruskal-Wallis tests. For the comparison of swim speed, we filtered out time points in which cells were not moving or displayed speeds greater than 150 µm/second (likely measurement error).

> All **code** generated and used for the pub is available in this GitHub repository (DOI: 10.5281/zenodo.7015909), including Fiji macros, Cellprofiler project files, and R analysis files.
>
> Associated **imaging datasets** are available in these Zenodo repos: *C. steinii* cyst rotation, early encystment, in a 384-well plate or microwells, and reanimation; and *Tetrahymena rostrata* swimming.

Key takeaways

We have engineered microchambers to confine swimming cells within a static field of view during time-lapse microscopy, without perturbing cell behavior. The microchambers increase the probability of a cell being in the field of view, such that 30–40% of the total cells in the chamber are in focus at any given time. This is an improvement compared to a standard imaging chamber (384-well plate) where only ~2% of total cells are in the field of view at any given time.

The microchambers are constructed with 3D-printed parts and commercially available components. Researchers can use the microchambers to observe swimming cells or cells across life cycle changes over extended periods of time.

Next steps

We can potentially make further improvements to increase the probability of a cell being in the field of view even more than 40% of the time by reducing the depth of the imaging chamber. Currently, the depth of the chambers, which is defined by the thickness of the 3D-printed spacer, is 600 µm. It's possible to print a spacer as small as 100 µm with a stereolithography 3D printer. Evaporation becomes a challenge when using small-volume microchambers. Aliquoting in a humid environment is one solution to evaporation from the chambers. Since releasing the first version of this pub, we developed a new strategy to confine cells, this time using stamps to mold small microchambers into agar **[7]**.

Ultimately, we will use these microchambers to study protists, such as *Colpoda steinii*, at various stages of their complex life cycles, such as the reanimation of resting cysts to an active state.

We're interested in learning whether this resource would be useful to other scientists or for other applications. We're also interested in learning whether there are simpler or easier-to-use methods for confining highly motile cells during time-lapse imaging. Please don't hesitate to share your thoughts in a comment — we'd love the feedback.

Acknowledgements Thank you to Mert Celebi for helping to think through and test different options for sharing and archiving code.

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