A high-throughput imaging approach to track and quantify single-cell swimming

Live imaging of swimming cells can yield insight into an organism's viability and responses to environmental stimuli. We developed a microscopy workflow and image analysis pipeline, SwimTracker, to track motility phenotypes from swimming cells in high throughput.

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Purpose

We need robust, high-throughput methods to observe and quantify biology across species. Historically, quantitative measurement of single-cell motility, even at low throughput, has proven challenging partly due to the difficulty of isolating cells [1]. We previously addressed the issue of cell isolation using agar microchambers [2], an effective but low-throughput method for observing long swimming trajectories of cells. Here, we develop a new single-cell motility data acquisition and analysis workflow (SwimTracker) that increases the throughput and versatility of our previous sample preparation approach (microchambers), and we demonstrate its application to more sample preparation methods (e.g., swimming in microtiter plates). We show that this approach enables robust quantitative readouts of motility even without isolating single cells.

We developed this strategy by 1) scaling image acquisition using the automation capabilities of our commercial microscope software, 2) directly comparing two types of vessels (agar microchambers and 384-well microtiter plates) to increase the flexibility of the assay, and 3) streamlining and automating the cell tracking and statistical analyses to make the assay robust and high-throughput.

This resource should be helpful for researchers studying motility in unicellular and small multicellular organisms. Our approach allows for extremely high throughput analysis of single-cell motility data (10s of thousands of cells) even without isolating single cells.

- This pub is part of the **platform effort**, "Microscopy: Visually interrogating the natural world." Visit the platform narrative for more background and context.
- All associated **code** for tracking cell trajectories, calculating motility metrics, and conducting statistical analysis (the **SwimTracker** pipeline) is available in this GitHub repository.
- All **data**, including the raw time-lapse microscopy data and computed cell trajectories, is available via the BioImage Archive.

The strategy

We're using microscopy to capture phenotypes at high throughput. In this work, we focus on motility, an evolutionarily conserved, information-rich readout impacted by many biological processes, including life stage, metabolism, and physical and sensory interactions with the environment [3][4][5]. Motility is common to multicellular and unicellular organisms [6][7] and takes diverse forms (e.g., walking, jumping, gliding, crawling, etc.). We're focused on motility in liquid (swimming), a form of movement common to many protists, an evolutionarily diverse and under-characterized taxonomic group that is the focus of many studies at Arcadia [8]. Thus, we needed a flexible and easy method to capture motility phenotypes in high throughput across many species and environments.

The problem: Current methods for acquiring and analyzing motility data don't scale

Our prior approach to *in vivo* imaging, which let us track individual cells, used agar microchambers to isolate cells. This works especially well for capturing long swimming trajectories but doesn't efficiently scale either to many cells (hundreds to thousands) or many different species and conditions because each differing group requires its own agar pad. In addition, our previous motility analysis workflow is likely too slow for the large dataset size required for our future high-throughput analyses.

Our solution: A streamlined workflow to acquire, process, and analyze microscopy videos to study motility

We created a method to 1) capture cell trajectories from many pooled individuals, 2) extract features of their movement, and 3) compare motility metrics across groups (Figure 1). Our workflow acquires 20-second brightfield videos at 20 frames per second and then quantifies features of swimming in single-celled organisms. We increased the data acquisition by loading cells in microtiter plates and by automating the acquisition of time-lapse microscopy videos using Nikon's NIS-Elements software (JOBS) software.

The bulk of this resource is a computational pipeline ("SwimTracker") for segmentation, cell tracking, and extracting motility metrics from the time-lapse microscopy videos (Figure 1). In addition, we generated a set of Jupyter notebooks that let you aggregate these summary motility metrics, statistically compare them across different populations of cells, and visualize differences between them via univariate and multivariate analysis (Figure 1).

We also describe how we applied this strategy to measure swimming in the unicellular alga *Chlamydomonas reinhardtii* and validated it regarding imaging time, sample preparation, and imaging vessel. We less rigorously tested but also validated that we could track swimming in organisms smaller (5 µm) and much larger (125 µm) than 8 µm-long *Chlamydomonas* (Supplemental Figure 1 and Figure 2).

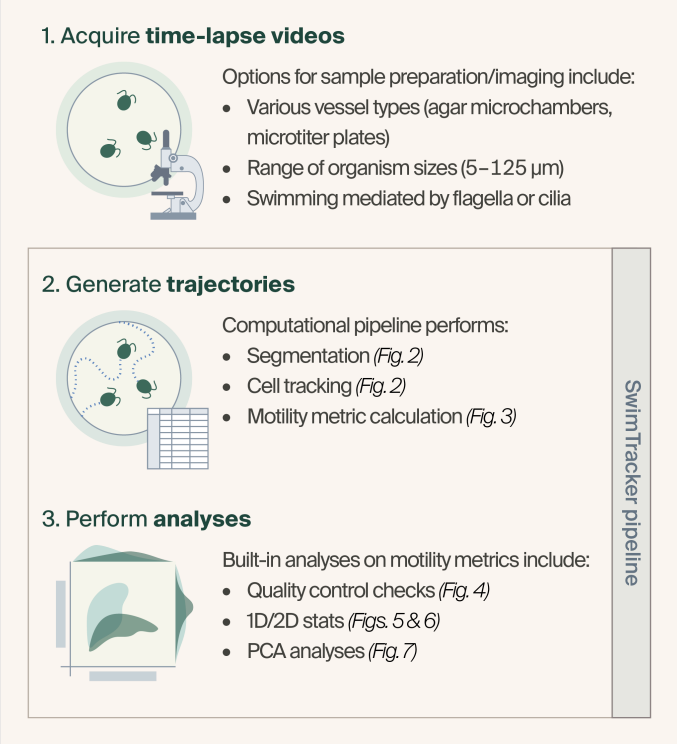


Figure 1. **Overview of our approach to high-throughput motility data acquisition and analysis**.

We've highlighted features of the SwimTracker computational pipeline and its compatibility with a range of sample preparation options for imaging.

These *in vivo* imaging methods let you quantitatively compare diverse swimming phenotypes across groups of interest. We think they'll be relevant for researchers interested in understanding the mechanisms of movement, such as ciliary/flagellar beating and the responses of protists to drugs and other stimuli. The high throughput enabled by our approach also allows the study of many species and environmental conditions.

The approach

To develop this swimming assay for single-celled organisms, we first established an automated protocol for recording time-lapse microscopy videos (see "Microscopy") using a unicellular algae *Chlamydomonas reinhardtii* (see "Cell culture and preparation for imaging") in two different sample formats (see "Vessel

preparation"). The two formats, or vessels, were agar microchambers (we refer to these as "microchambers") and individual wells of a 384-well microtiter plate (we refer to these as "wells"). We compared these two sample formats to see whether one might be better for particular use cases, potentially increasing flexibility for the user (spoiler: they both work, but format really matters. More info on that in "Vessel type strongly impacts motility").

Sample preparation

Cell culture and preparation for imaging

We ordered wild-type *Chlamydomonas reinhardtii* strain CC-124 from the Chlamydomonas Resource Center (University of Minnesota). After receiving the strain from the culture center, we prepared lawn plates as described previously [9]. We maintained clonal populations from stock streaks by live transfer once every two weeks on 1.5% agar plates with tris-acetate-phosphate (TAP) medium. We incubated plates at room temperature under 12:12 light: dark cycles. For all motility experiments, we transferred cells (1 cm strip using a loop) from lawn plates to water to induce the mixed-stage cells to become gametes, which are flagellated and motile [10][11]. We resuspended these cells in water by agitating the loop against the inside wall of a 1.5 mL microcentrifuge tube containing 500 µL of sterile Milli-Q water. We wanted to test factors influencing the proportion of cells that became gametes. To do this, we left tubes on the bench for 4 or 21 h to compare populations of cells that spent different amounts of time in water and might be at different stages of transition to gametes. We also compared cells that we pipetted from either the topmost portion of the water or the middle but above the pellet of settled cells.

We ordered wild-type *Isochrysis galbana* strain UTEX987 from the Culture Collection of Algae at The University of Texas at Austin. After receiving the strain, we grew 200 mL liquid cultures in Erdschreiber's medium on an orbital shaker at 120 rpm at room temperature under 12:12 light: dark cycles. We diluted cells two-fold in synthetic seawater.

We ordered wild-type *Paramecium tetraurelia* strain 8s 4-d2 from the Culture Collection of Algae and Protozoa. After receiving the strain, we prepared liquid cultures in Chalkey's medium pre-seeded with *C. reinhardtii* strain CC-124 as a

food source. We maintained 10 mL cultures on the bench at room temperature without shaking in T75 cell culture flasks.

Vessel preparation

We imaged cells in two types of vessels — agar microchambers and microtiter plates (Figure 2).

We made agar microchambers using a PDMS stamp purchased from RMS Microstamps [2], following our protocol, "Molding microchambers in agar with PDMS stamps for live imaging" [12]. The dimensions of the stamp's protrusions resulted in circular indents in the agar that were 100 µm in diameter and 40 µm deep. Because these microchambers are so small, shallow, and numerous (~10,000 for a stamp ⅞ in^2), pipetting directly into individual wells is impossible; therefore, we load a single strain or species into each individual stamped piece of agar.

To prepare samples on agar microchambers, we wetted the surface with 5–10 µL of water and then added 2 µL of cells onto one section of the agar at a time. We allowed the drop to spread across the agar and then visually checked the distribution of cells across the microchambers using an Olympus CK 2 inverted phase microscope. We repeated this process 2–5 times to ensure the cell density was somewhat evenly distributed across the microchambers. Before placing the coverslip (#1.5 thickness for imaging), we used a Kimwipe to wick up water at the edge of the agar and glass. Finally, we sealed the coverslips using a small paintbrush to apply VALAP (1:1:1 mixture of vaseline, lanolin, and paraffin) heated to 70 °C.

To load microtiter plates with either *C. reinhardtii* or *Isochrysis galbana*, we pipetted 20 µL of cells into the bottom of a well of a 384-well, black-walled, glass-bottom plate (Cellvis, #P384-1.5H-N). To pipette *Paramecium tetraurelia*, we first poured organisms into a 12-well plate. We visualized them on a phase contrast microscope before gently pipetting them using a wide-bore pipette tip (Molecular BioProducts, ART 200G) and transferring them into wells of the 384-well plate described above.

Microscopy

Hardware: Objective, microscopes, cameras

The preferred imaging setup differs depending on whether the cells have been loaded into agar microchambers or microtiter plates. Therefore, we performed brightfield time-lapse imaging on two different microscopes. For samples in agar microchambers, we imaged using an upright Nikon Ni-E microscope equipped with a Photometrics Kinetix digital sCMOS camera and built-in LED light source. We imaged samples in glass-bottom microtiter plates using an inverted Nikon Ti2-E & Yokogawa CSU W1-SoRa confocal microscope fitted with an ORCA-Fusion BT digital sCMOS camera (Hamamatsu) and a LIDA Light Engine (Lumencor) for illumination. However, this imaging could be done with any inverted widefield microscope and camera. We used the same type of objective lens (Nikon Plan Apo 10× 0.45 Air objective) for both microscopes. For both microscopes, we acquired data using the same software: Nikon NIS-Elements AR (version 5.42.03) and the "High-Content Analysis" package to implement automation.

Image acquisition parameters

The parameters we used for acquiring image data were:

- 20 s time-lapses recorded in brightfield at the rate of 20 frames per second (50 ms exposure time) with a 10× 0.45 NA air objective
- Light intensity set to maximize the dynamic range of the acquisition system
- 610 nm longpass filter (ThorLabs FGL610S) placed over the light source of the upright microscope [1]
- To prevent phototaxis, we imaged cells using red light as described previously [2]

We used these parameters to acquire videos on both the upright Ni-E widefield microscope (using agar microchambers) and the inverted Ti2-E microscope (using microtiter plates).

Automated acquisition workflow

We increased throughput by automating time-lapse microscopy acquisitions. We developed the automation workflows using Nikon NIS-Elements JOBS automation software and provide them on GitHub. While these workflows can only

immediately be implemented with compatible hardware and software, most modern microscopy software packages offer the same functionality. The workflow consists of the following steps:

1. **Define the optical configuration**: Set optical parameters such as the objective lens magnification, light intensity, exposure time, and time-lapse duration.
2. **Define the stage area**: Set the bounds of the translation stage to either the limits of the slide area containing agar microchambers or to match the geometry of the microtiter plate. If possible, define a focus surface to compensate for sample tilt as the stage is translated across the sample.
3. **Define a tiling scheme**: Create a grid of tiles that'll encompass the defined stage area. For imaging cells in microchamber pools, we generally set the field of view to contain 16 pools and tile with a small amount (1–2%) of overlap. For imaging cells in microtiter plates, we generally acquire one time-lapse per well. However, depending on cell density, it might be better to capture multiple fields of view per well.
4. **Run the acquisition**: For each field of view defined by the tiling scheme, acquire a time-lapse with the chosen optical configuration.

The resource

We're sharing an approach we developed to quantify swimming in small organisms using a computational pipeline, SwimTracker. While we also focus on the sample preparation used for measuring swimming unicellular algae, this part of the workflow is flexible — the rest works using any time-lapse data as input (Figure 1). SwimTracker takes raw time-lapse microscopy data of swimming cells, applies cell tracking, and outputs comma-separated value (CSV) files with extracted motility metrics and MP4 videos with animated trajectories of the tracked cells. Our GitHub repo for SwimTracker also includes a set of Jupyter notebooks for performing multidimensional analysis and statistical tests on the data.

In "SwimTracker tracks cells and measures a suite of motility metrics," we give an overview of our assay and the statistics SwimTracker calculates. In "Validating our strategy," we walk through quality-control checks we ran to ensure our results weren't affected by some obvious potential variables. While some of the variables

we checked had little impact or were somewhat specific to testing the workflow on *C. reinhardtii*, it's worth noting that we found the biggest differences in our calculated motility metrics based on the vessels in which we confined cells for imaging. We discuss these tests and recommendations for when to use different vessel types in "Vessel type strongly impacts motility."

SwimTracker tracks cells and measures a suite of motility metrics

We primarily used *Chlamydomonas reinhardtii*, a motile, unicellular alga, to develop this method. We prepared *C. reinhardtii* gametes in two types of vessels for brightfield imaging: agar microchambers and 384-well plates (see "Sample preparation").

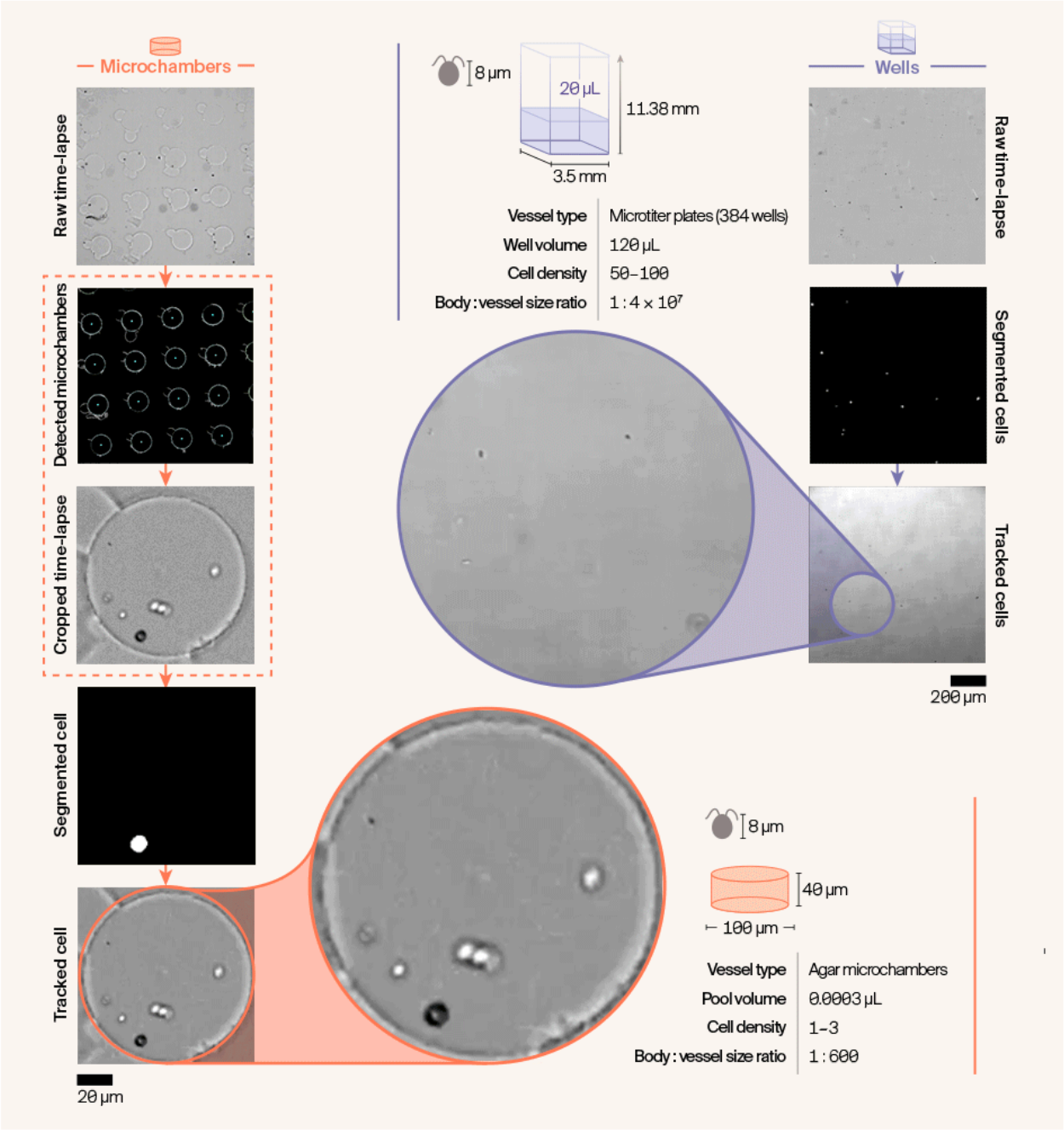


Figure 2. **A schematic of the image-processing pipeline for organisms swimming in agar microchambers (left, orange) and wells of glass-bottom microtiter plates (right, purple).**

We don't need to detect microchambers or spatially crop the time-lapse video for trajectories in wells.

We developed "SwimTracker" as a Python-based image processing pipeline to calculate cell trajectories from the time-lapse videos. It processes images of samples in agar microchambers (Figure 2, left) with two additional steps than the workflow for microtiter plates (Figure 2, right). These additional steps are necessary to detect and crop individual microchambers before cell segmentation and trajectory calculation. The pipeline detects individual microchambers by first

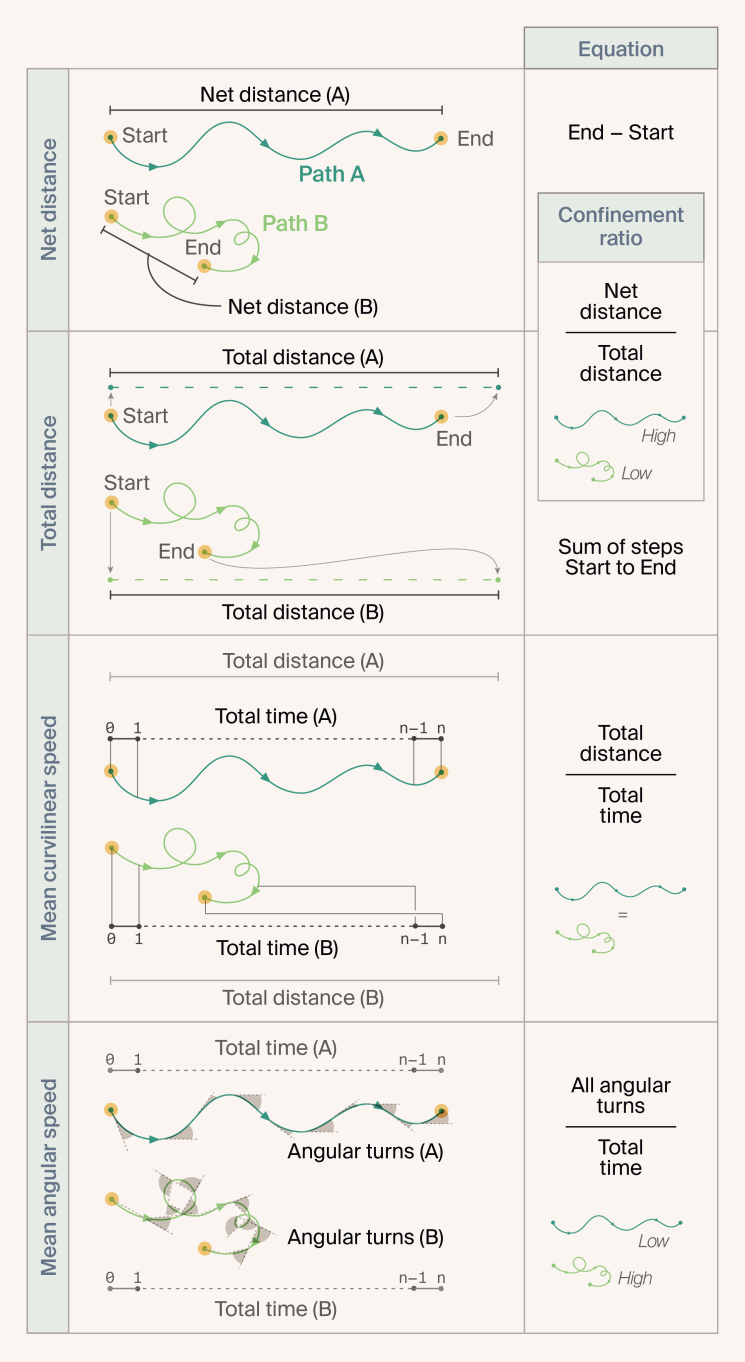


Figure 3. **Illustrations to visualize cell trajectory quantifications from time-lapse microscopy data**.

applying a Sobel edge filter to the mean intensity projection of the raw time-lapse. It then applies a Hough transform to the edge-enhanced image so we can identify and locate individual microchambers. SwimTracker then extrapolates this uniform, grid-like arrangement of microchambers to determine the locations of microchambers that the initial segmentation doesn't detect.

Next, SwimTracker segments cells from the time-lapse to facilitate cell tracking. First, it subtracts the mean intensity projection from each time-lapse to remove the static background and enhance the contrast of the cells. Then, the pipeline binarizes the videos using thresholding (Otsu's method, Figure 2, "Segmented cell(s)"). Finally, it tracks cells using btrack (version 0.6.5) [13] with the default configurations. SwimTracker outputs the trajectories of each segmented cell in CSV format for subsequent analysis.

To quantify swimming behavior, we calculated 11 metrics that capture various aspects of a unicellular organism's movement based on existing methodologies [14]. We describe all 11 metrics in Table 1, six of which we illustrate in Figure 3.

Motility metric	Description
Total time*	Total time of cell trajectory
Total distance*	Total distance traveled along a trajectory
Net distance*	Distance between the start and end point of the trajectory
Max sprint length	Maximum distance traveled in a given time interval
Confinement ratio*	The ratio of net distance to the total distance
Mean curvilinear speed*	The average speed of a cell along its curved trajectory
Mean linear speed	Average speed of a cell along a straight path between its start and end point
Mean angular speed*	Average rate of angular change
Number of rotations	Number of rotations a cell makes along its trajectory
Number of direction changes	Number of times a cell changes its direction minus the total number of sign changes in its velocity
Pivot rate	The ratio of the number of direction changes to the total distance

Table 1. **Table of motility metrics that SwimTracker calculates to characterize a cell's trajectory**.

Metrics with asterisks are illustrated in Figure 3.

Raw trajectories require filtering

In our trajectory data, some traces of single cells were clearly problematic. For example, in wells, many cells swim in and out of the focal plane, leading to many short trajectories and the possibility of capturing more than one trajectory per cell. Furthermore, non-motile or minimally motile cells in both wells and microchambers can lead to trajectories with long temporal duration but little spatial displacement. To eliminate trajectories from minimally motile cells and reduce the likelihood that we were analyzing more than one trajectory per cell, we filtered trajectories to be at least 10 s in duration (total time) and 20 µm in length (total distance). Both filters are implemented in "1_compute-summary-motility-metrics.ipynb."

Validating our strategy

We performed a series of tests to evaluate the impact of experimentally controllable parameters on the data acquisition and analysis workflow. First, we tested whether our imaging parameters (e.g., duration, temporal sampling density, light exposure) affected swimming behavior. Next, because we wanted to apply this approach to assay gametic swimming, we examined two experimental factors influencing the life history transition to gametes. Finally, we tested whether different vessels produce different swimming statistics.

Imaging time doesn't affect swimming

We wanted to image as briefly as possible to enable large-scale data acquisition across organisms or samples, but acquisition time could impact the motility statistics. Therefore, we assessed whether motility measures changed across the acquisition period (Figure 4, "2_temporal-variation-in-motility-metrics.ipynb"). We computed linear regressions between each individual metric and image acquisition time to calculate correlation coefficients for these relationships (Figure 4, Supplemental Table 1). Ten of the eleven metrics weren't correlated with imaging time ($p > 0.1$ in all cases, linear regression, Supplemental Table 1), the exception being max sprint length, which weakly correlated ($p = 0.048$, Supplemental Table 1). In the scatter plots below, we highlight the three metrics we focus on for the rest of the analyses (Figure 4). Overall, we found that on these timescales, the swimming behavior of the cells isn't influenced by the imaging duration.

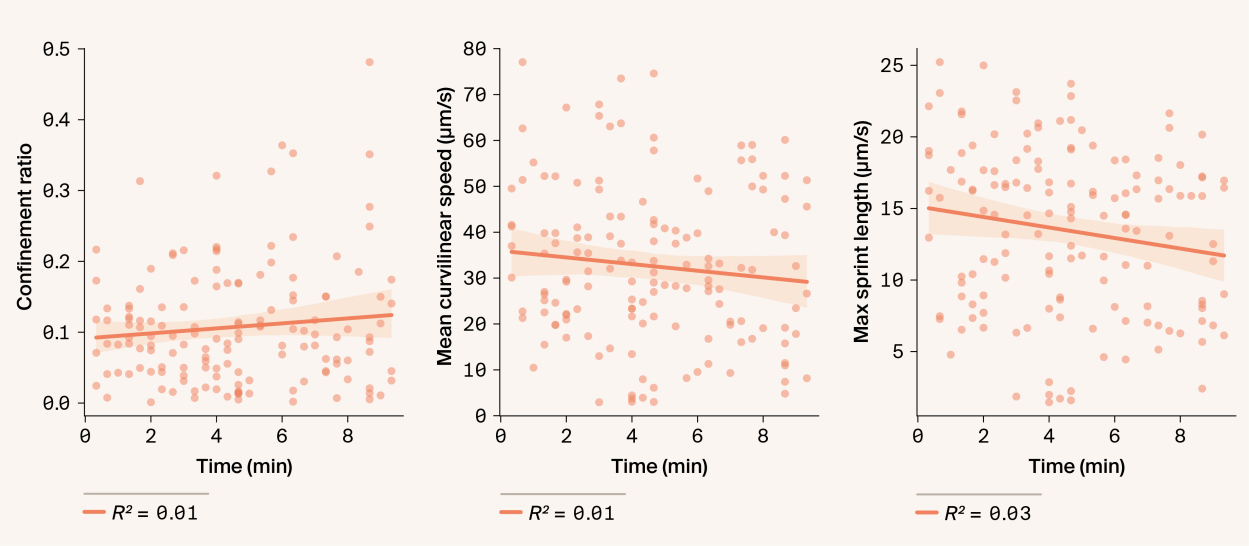


Figure 4. **Scatter plots showing the variation of several motility metrics over the duration of an imaging experiment in microchambers**.

Each point in the scatter plot corresponds to an individual cell's trajectory at a particular 20 s interval during the experiment. Linear regressions show no significant correlation between imaging duration and confinement ratio and mean curvilinear speed ($p > 0.1$, linear regression) but a slight correlation with max sprint length ($p < 0.05$, linear regression). Note that we translated the stage between each time-lapse such that in each 20 s time interval, we imaged a different group of cells. Multiple points exist at each 20 s interval in the scatter plots because each field of view contains multiple microchambers.

SwimTracker can capture subtle effects on motility

We wanted to evaluate SwimTracker's ability to capture subtle changes in motility. We've worked extensively with the single-celled alga *Chlamydomonas reinhardtii* and have previously used differing sample preparations to alter its motility [9]. We, therefore, evaluated the influence of two factors on motility. We include the data here not to focus on the results but to show an example of the type of univariate comparisons that SwimTracker can quickly generate.

First, we induced gametogenesis in actively growing vegetative populations for differing amounts of time (either 4 h or 21 h [10][11]) with the expectation that longer induction will result in more gametes in the population. Gametes have motility that differs from vegetative cells [4]. Second, following induction, we collected cells from the top of the water column and cells from the middle of the water column, expecting that the more motile cells would be higher in the water column. We then evaluated whether SwimTracker could capture the expected differences in motility.

We found that while neither the position in the tube nor time in water significantly impacted the mean angular speed or confinement ratio, they both influenced the mean curvilinear speed (Figure 5). Cells we pipetted from the top of the tube were 17% faster (as indicated by the mean curvilinear speed) than those from the middle (p = 0.046, Mann–Whitney U) (Figure 5, A). Moreover, cells that spent only four hours in water swam on average 24% faster than those that spent 21 h in water (p = 0.039, Mann–Whitney U) (Figure 5, B). These results showed that both the position of cells in the tube and the time spent in water can affect motility. This was unsurprising, but shows how SwimTracker can be used for 1-dimensional comparisons between variables of interest.

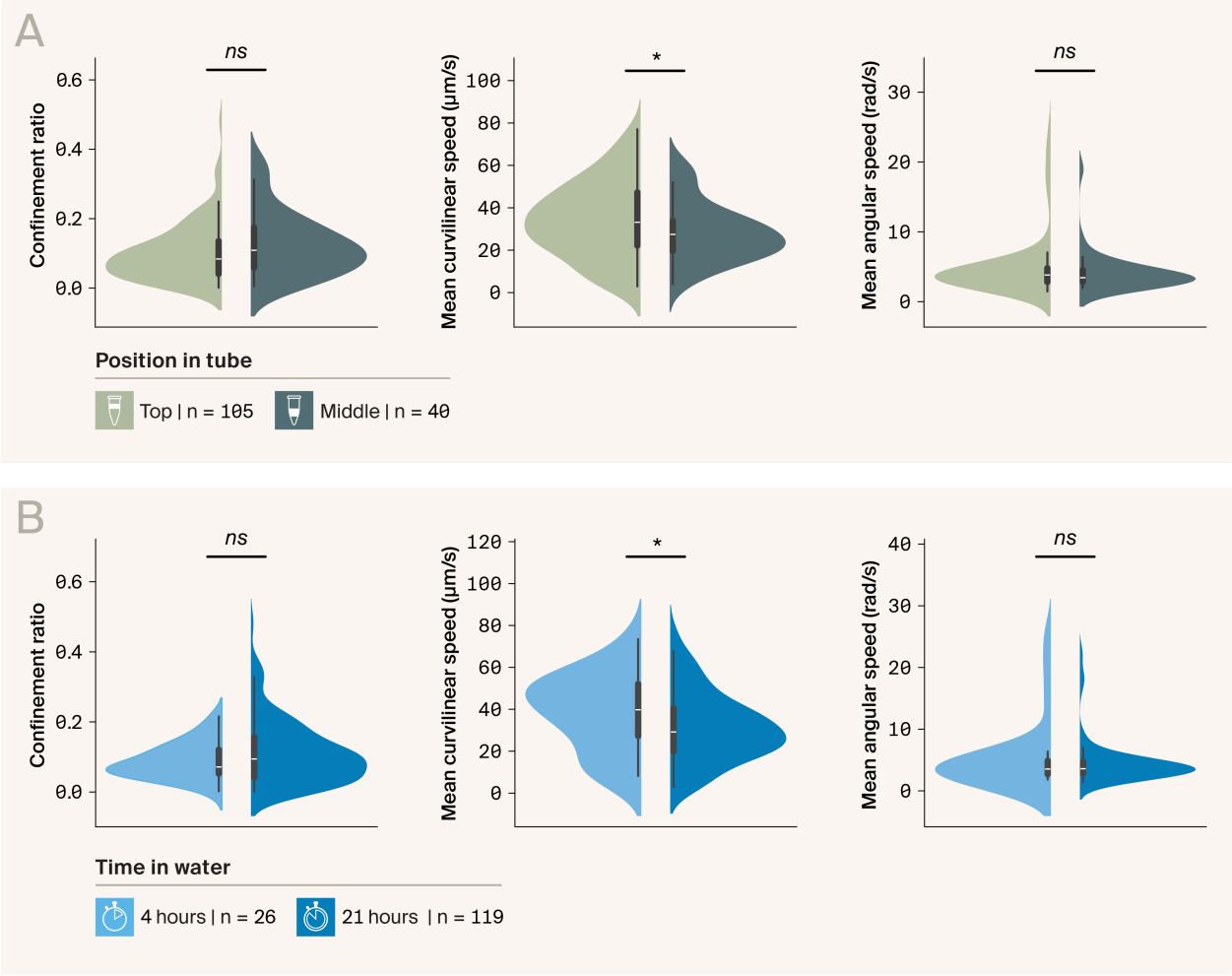


Figure 5. **Kernel density estimates of motility metrics for cells grouped by different experimental variables.**

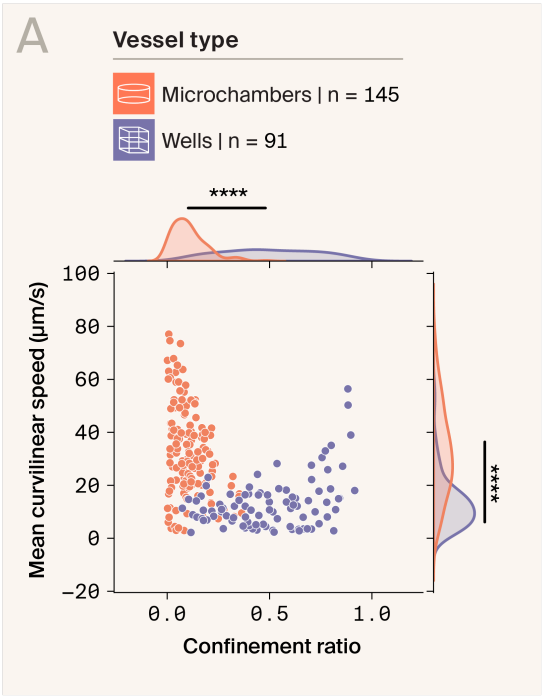
(A) Distributions of confinement ratio, mean curvilinear speed, and mean angular speed for cells we pipetted from the top of the tube (light green) versus the middle of the tube (dark green).

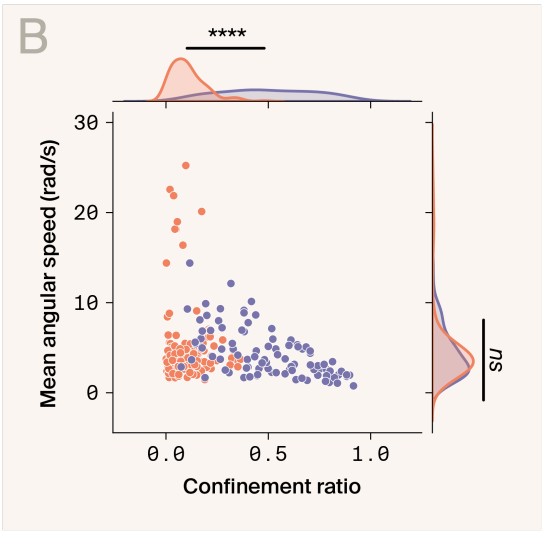
(B) Distributions of the same metrics for cells that spent 4 h in water (light blue) vs. 21 h in water (dark blue) before imaging.

Statistical significance: * indicates $p \leq 0.05$; ns indicates $p > 0.05$ as determined by Mann–Whitney *U* tests.

Vessel type strongly impacts motility

While we'd ideally collect motility data that reflects normal behavior in a realistic environment, vessel type may impact the motility we're measuring. Therefore, we examined the effect of the sample format (the “vessel”) on cell swimming (see “Vessel preparation” in Sample Preparation). Our goal was to test whether cells behave differently in the two types of vessels, agar microchambers vs. microtiter plate wells, which differ in total volume, confinement, and the number of cells they can accommodate (Figure 2). While the agar microchambers [2] are extremely useful for imaging many cells of a single species, the sample preparation is


A
Vessel type
Microchambers | n = 145
Wells | n = 91
****
Mean curvilinear speed (µm/s)
100
80
60
40
20
0
−20
0.0
0.5
1.0
Confinement ratio
****


B
****
Mean angular speed (rad/s)
30
20
10
0
0.0
0.5
1.0
Confinement ratio
ns

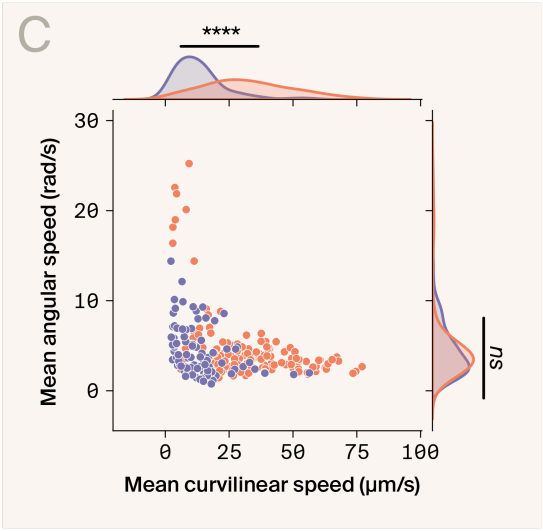

C
****
Mean angular speed (rad/s)
30
20
10
0
0
25
50
75
100
Mean curvilinear speed (µm/s)
ns

Figure 6. **The choice of vessel type influences the distribution of certain motility metrics**.

The distributions of the confinement ratio and mean curvilinear speed are much narrower for pools than for wells, while the mean angular speed appears less impacted.

**** indicates $p < 0.001$ and ns indicates $p > 0.05$, Mann–Whitney *U*.

laborious and difficult to apply to many conditions or strains. Some of our future motility work requires comparisons between many treatments, so we wanted to see if we could quantify cell trajectories in microtiter plates.

We found that swimming behavior in microchambers differed substantially from that in wells of microtiter plates. We compared confinement ratio, mean curvilinear speed, and mean angular speed from cell trajectories in microchambers to those in wells (Figure 6). We expected that the confinement ratio, which is the net distance of a cell track divided by the total distance, would vary between microchambers and wells because of their differing physical dimensions (Figure 2). In line with this expectation, we found that the cell movement was more confined in microchambers (lower confinement ratio) than cells in wells (microchambers: 0.11 ± 0.09; wells: 0.50 ± 0.23; Mann–Whitney *U*, $p < 0.001$) (Figure 6, A and B).

We compared mean curvilinear speeds and found that cells were > 2× faster on average in microchambers than cells in wells (microchambers: 33 ± 17 µm/s; wells: 13 ± 10 µm/s; Mann–Whitney *U*, $p < 0.001$) (Figure 6, A and C). A previous study examined the effect of microchamber size on *C. reinhardtii* swimming using microfluidics. They varied the diameter but not the height of their trap sizes and found that cells swam faster in wider traps (200 µm diameter, 30 µm height) [1]. The vessel types that we examined varied from each other in not only x and y dimensions but also in z, resulting in substantial differences in volumes (see Figure 2).

We observed no effect of vessel type on mean angular speed (Figure 6, B and C). Taken together, these results demonstrate that the choice of vessel type influences the distribution of measurements for certain aspects of swimming, highlighting the critical importance of selecting the correct sample preparation method for the specific experimental task.

While the two-dimensional plots were informative, we wanted a more holistic sense of motility differences without selecting the metrics to describe them *a priori*. We performed a principal component analysis (PCA) on six metrics (Figure 7, Table 1). We selected these six metrics because they're ratio-based and not biased by trajectory duration. Because of the limited depth of focus, the trajectories we obtained from microchambers have a longer duration, on average, than those from cells in wells. This PCA analysis revealed a separation between microchamber and well trajectories with limited overlap in PC 1 (Figure 7, A). This component's most heavily weighted features are max sprint length, confinement ratio, and mean curvilinear speed (Figure 7, B). This suggests that these two types of motility differ in speed and amount of turning, which is consistent with our analysis of the individual metrics. And these two classes of trajectories can be almost completely separated based on our metrics (Figure 7, A; PC1).

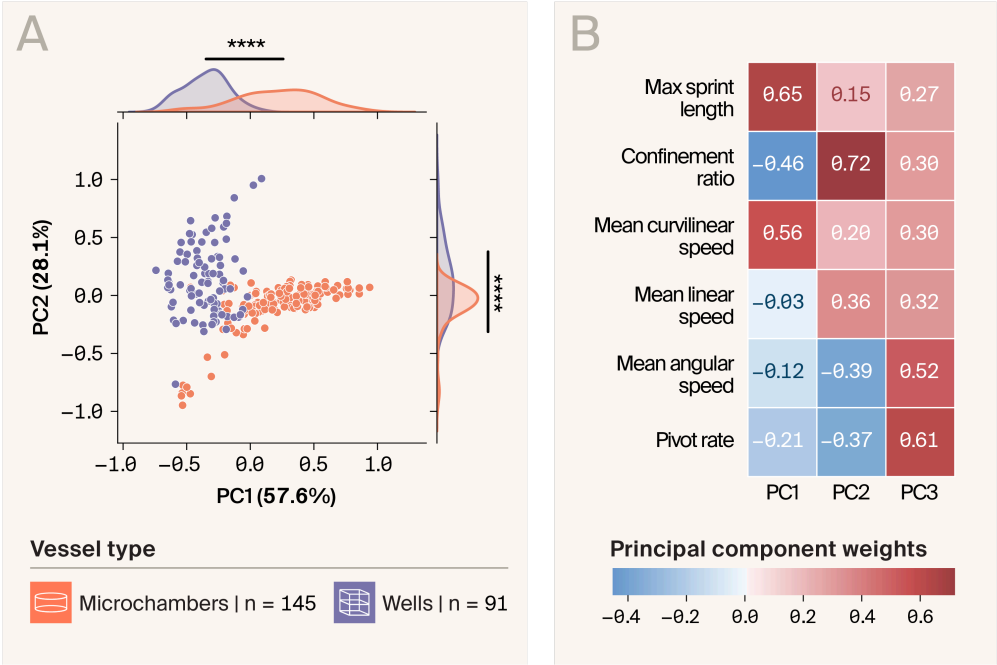


Figure 7. **Principal component analysis (PCA) on motility metrics of *Chlamydomonas reinhardtii* swimming in two different vessels.**

(A) Separation of trajectories between groups as a function of the first two principal components. (B) The weights for each of the six motility metrics are included in the PCA. The first PC seems to discriminate the trajectories based on how fast and straight they are, while the second PC is dominated by confinement.

Taken together, these results demonstrate that our workflow lets us analyze motility for a much greater number and diversity of samples and allows us to

distinguish subtle behavioral differences across experimental conditions.

Additional methods

We used ChatGPT to suggest wording ideas and then chose which small phrases or sentence structure ideas to use. We also used ChatGPT to help clarify and streamline text that we wrote. Additionally, we used Grammarly Premium to help copy-edit draft text to match Arcadia's style and to help clarify and streamline text that we wrote.

Key takeaways

Our computational pipeline, SwimTracker, lets you quantify swimming trajectories of single-celled organisms from time-lapse microscopy datasets in high throughput. If you aim to acquire high-resolution, single-cell tracks of only a few types of cells for extended periods of time, agar microchambers are optimal. However, if cell trajectories of a population of cells are sufficient and your goal is to compare many treatments, then microtiter plates are best.

Takeaways

1. SwimTracker works on brightfield microscopy videos to quantify a suite of motility metrics for single cells that swim.
2. SwimTracker works on isolated cells (in agar microchambers) and groups of cells (in microtiter plates).
3. Choosing a vessel type for organisms can influence the throughput of the assay and even cell swimming behavior.

Next steps

We plan to use SwimTracker to compare motility from populations of *Chlamydomonas* algae with different genetic backgrounds (e.g., hybrid progeny

from a genetic cross, as well as mutant strains that model specific diseases) and under different environmental parameters (e.g., nutrients, drug treatments).

In the future, we'll use data-adaptive and machine-learning-based approaches to classify cell motility behavior, allowing us to rapidly identify environmental or genetic parameters impacting motility.

We did some preliminary work to confirm that this imaging workflow could work on organisms of various sizes, including organisms both smaller (5 µm) (Supplemental Figure 1) and larger (125 µm) (Supplemental Figure 2) than *C. reinhardtii* (8 µm). We'd love to hear how our approach works as a motility assay for swimming organisms within or beyond that size range and whether you can adapt it for other types of locomotion (e.g., crawling, gliding, etc).

Acknowledgments

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Contributors (A–Z)

- **Audrey Bell**: Visualization
- **Keith Cheveralls**: Validation
- **Tara Essock-Burns**: Formal Analysis, Investigation, Visualization, Writing
- **Megan L. Hochstrasser**: Editing
- **Ryan Lane**: Formal Analysis, Investigation, Software, Visualization
- **David G. Mets**: Supervision

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