Computer-Assisted Tracking of Chlamydomonas Species

Alexandra M. Folcik¹, Timothy Haire¹, Kirstin Cutshaw¹, Melissa Riddle¹, Catherine Shola¹, Sararose Nassani¹, Paul Rice¹, Brianna Richardson², Pooja Shah¹, Nezamoddin Nazamoddini-Kachouie³ and Andrew Palmer¹,4,5*¹

¹Department of Biomedical and Chemical Engineering and Sciences, Florida Institute of Technology, Melbourne, FL, United States, ²Department of Aerospace, Physics, and Space Sciences, Florida Institute of Technology, Melbourne, FL, United States, ³Department of Mathematical Sciences, Florida Institute of Technology, Melbourne, FL, United States, ⁴Department of Ocean Engineering and Marine Sciences, Florida Institute of Technology, Melbourne, FL, United States, ⁵Aldrin Space Institute, Florida Institute of Technology, Melbourne, FL, United States

The green algae Chlamydomonas reinhardtii is a model system for motility in unicellular organisms. Photo-, gravi-, and chemotaxis have previously been associated with C. reinhardtii, and observing the extent of these responses within a population of cells is crucial for refining our understanding of how this organism responds to changing environmental conditions. However, manually tracking and modeling a statistically viable number of samples of these microorganisms is an unreasonable task. We hypothesized that automated particle tracking systems are now sufficiently advanced to effectively characterize such populations. Here, we present an automated method to observe C. reinhardtii motility that allows us to identify individual cells as well as global information on direction, speed, and size. Nutrient availability effects on wild-type C. reinhardtii swimming speeds, as well as changes in speed and directionality in response to light, were characterized using this method. We also provide for the first time the swimming speeds of several motility-deficient mutant lines. While our present effort is focused around the unicellular green algae, C. reinhardtii, we confirm the general utility of this approach using Chlamydomonas moewusii, another member of this genus which contains over 300 species. Our work provides new tools for evaluating and modeling motility in this model organism and establishes the methodology for conducting similar experiments on other unicellular microorganisms.

Keywords: Chlamydomonas reinhardtii, automated tracking, algae motility, chemical biology, phototaxis, Chlamydomonas moewusii

INTRODUCTION

The unicellular alga Chlamydomonas reinhardtii is a model organism for the study of flagellar motility, photosynthesis, and a variety of biotechnology applications among unicellular eukaryotes. This photoautotroph has minimal culture requirements, is genetically tractable, and has an extensive strain repository including numerous motility mutants (Luck et al., 1977; Huang et al., 1981; Huang et al., 1982a; Huang et al., 1982b; Kuchka and Jarvik, 1982; Segal et al., 1984; Kamiya, 1988; Barsel et al., 1988; Bloodgood and Salomonsky, 1989; Kuchka and Jarvik, 1987; Kamiya et al., 1991). Photo-, gravi-, and chemotaxis have all been associated with this organism, making it an ideal system for understanding how multiple inputs can be integrated to regulate motility in unicellular organisms. Prior efforts...
to quantify \textit{Chlamydomonas} motility have largely focused on high-speed photographic evidence, which was then analyzed by manual tracking (Racey et al., 1981). However, such methods are time-consuming, prone to error, and unlikely to resolve multiple responses within a population. Moreover, algal cultures frequently reach densities ranging from $10^3$ to $10^5$ cells/ml. The results of a handful of tracks (<30) are unlikely to be an accurate reflection of the behavior of such a population. The ability to observe a larger sample size would also provide refined insight into the dynamic response range of this model organism rather than just an average.

Automated particle tracking in which individual constituents of a population, biotic or abiotic, are followed over time can yield data on particle speed, directionality, size, and other features of interest for understanding the behavior of the observed population. In the case of \textit{C. reinhardtii}, we hypothesized that such approaches would be able to accommodate significantly more tracks, providing better models of behavior within a population of cells. Here, we report a new method to characterize motility in \textit{C. reinhardtii}, which allows us to identify individual particles (cells) as well as gather population-wide information on speed as well as directionality. The proposed strategy requires only a microscope with a camera to collect images and utilizes a publicly available software package, the TrackMate plugin for ImageJ, for analysis (Schneider et al., 2012; Tinevez et al., 2017).

In the present study, we evaluated the impacts of light and nutrient availability on motility in wild-type \textit{C. reinhardtii}, as well as characterizing a series of motility-deficient mutant lines. The ability to quantify the effects of such mutations provides a refined perspective on the impacts such mutations may have on these organisms. Our work provides a new tool for evaluating and modeling motility in this model organism. Furthermore, we confirm that the established methodology is able to characterize motility in another member of this genus, \textit{Chlamydomonas moewusii}, supporting the broader utility of this approach for observing motility in other unicellular microorganisms which may have important roles in host–microbial associations and/or biotechnology.

\section*{MATERIALS AND METHODS}

\subsection*{Materials}

Unless stated otherwise, all reagents were purchased from either Fisher Scientific or Sigma Aldrich.

\subsection*{Algae Growth And Media}

\textit{C. reinhardtii} wild-type (cc124), \textit{C. reinhardtii} mutants (see Table 1), and \textit{C. moewusii} (formerly \textit{Chlamydomonas eugametos}) were acquired from the Chlamydomonas Resource Center (http://www.chlamycollection.org/) (Luck et al., 1977; Huang et al., 1981; Huang et al., 1982a; Huang et al., 1982b; Kuchka and Jarvik, 1982; Segal et al., 1984; Kameya, 1988; Barsel et al., 1988; Bloodgood and Salomonsky, 1989; Kameya et al., 1991). Individual lines were maintained by streaking cultures on plates of Tris-acetate-phosphate (TAP) media with agar. Liquid cultures were prepared by growing lines individually in 25 ml of TAP media for 72 h under a 16:8-h day/night cycle (~13,800 W/m$^2$) at 23°C on a shaker (~150 rpm) (Haire et al., 2018). Minimal media studies were performed by limiting the amount of available nitrogen by restricting the amount of NH$_4$Cl, either to: low (50%, 400 mg/l) or NH$_4$-free TAP media.

\subsection*{Video Acquisition}

All motility data was acquired under minimal lighting in a darkroom to minimize background phototactic effects. Cell density was determined by fixing 200 µl aliquots of cells in 1.7% formaldehyde and manually counted using a hemocytometer at ×400. The remainder of the culture was incubated at 23°C in the dark for 30 min to reduce the impact of light on cell movement. Algae cultures were vortexed for ~30 s to resuspend any settled algae. Aliquots of 30 µl were placed on a slide and viewed under an Olympus CH30 binocular microscope at ×100 magnification. Cells were allowed to settle to avoid artificial movement of the cells caused by convection currents in the liquid on the slide. ToupView software (www.touptek.com) was used to collect videos with an AmScope FMA050 fixed microscope camera. Videos were collected with a frame rate of 7.5 frames/s for approximately 30 s.

\subsection*{Video Analysis}

Videos were imported into Fiji for splitting and tracking analysis (Schindelin et al., 2012). Calibration was done using a micrometer ruler slide to determine pixel length. To analyze tracks, the pre-installed Fiji plugin TrackMate was utilized (Tinevez et al., 2017). Cell size was also determined through Fiji. The resulting files were saved in comma-separated values (CSV) format as “Spots in tracks statistics,” “Track statistics,” and “Links in tracks statistics.” To determine the directionality of each track, the files “Spots in tracks statistics,” “Track statistics,” and “Links in tracks statistics” were combined using the statistical computing software R using the RStudio interface (www.rstudio.com) (R Development Core Team, 2016). This allowed for the creation of a new file in Tab delimited Text format containing direction vectors of all cell tracks. From the combined R file, Rose plots were generated by dividing the slide viewing area into eight wedges and plotting the total fraction of cells in each section. Preliminary findings were confirmed using the Chemotaxis and Migration Tool (www.ibidi.com). Histograms of varying speeds within the population were generated by the data analysis package in Excel. Average speeds of tracks were determined using the “Track statistics” file. Units were converted to micrometers per second by dividing the time of the video by the number of image slices.

\subsection*{Phototaxis Studies}

Light intensity studies were completed using a Leica 13410311 Illuminator dissection scope leveled with the microscope platform. This allowed for the light to pass perpendicular to the slide across the sample. Light intensity settings were measured using Digital Luxometer. Cultures of 30 µl were loaded onto slides and exposed to the indicated intensity of light immediately before data collection.

\section*{RESULTS}

\subsection*{Particle Tracking}

The digital equivalent of manual tracking across multiple video frames with hundreds of particles (cells) rapidly becomes
TABLE 1 | Chlamydomonas reinhardtii motility mutants, their mutation types, and average speeds (± standard error) as compared to wild-type (cc124).

| Strain ID | Mutation type | Avg. speed | % Wild-type | Reference |
|-----------|---------------|------------|-------------|-----------|
| cc124     | Wild-type     | 40.0 ± 4.5 | 100         | –         |
| cc125     | Phototactic aggregation | 46.0 ± 2.0 | 115         | Pröschold et al., 2005 |
| cc802 pf1 | No radial spoke heads | 2.8 ± 0.6 | 5           | Luck et al., 1977 |
| cc1026 pf3 | Axonemal protein defects | 9.0 ± 5.0 | 20          | Huang et al., 1982b |
| cc1032 pf14 | Lacks radial spokes | 3.5 ± 0.1 | 6           | Huang et al., 1981 |
| cc1036 pf18 | No central microtubules | 1.5 ± 0.7 | 3           | Bloodgood and Salomonsky, 1989 |
| cc1926 un1 | Single flagellum cells | 15.0 ± 3.0 | 38          | Huang et al., 1982a |
| cc2228 cda1 | Lacks outer dynein arms | 11.0 ± 2.0 | 23          | Kamiya, 1988 |
| cc2288 lt2-4 | Long flagella | 32.0 ± 1.0 | 80          | Barsel et al., 1988 |
| cc2530 vl2 | 0–6 flagella | 4.0 ± 2.0 | 10          | Kuchka and Jarvik, 1982 |
| cc2670 ida4 | Dynein arms | 14.0 ± 2.0 | 35          | Kamiya et al., 1991 |
| cc2679 mbt1 | Flagellar axoneme | 3.4 ± 0.6 | 9           | Segal et al., 1984 |
| cc3663 shf1 | Short flagella | 22.0 ± 2.25 | 50          | Kuchka and Jarvik, 1987 |

*Reference. #Average speed expressed in terms of micrometers per second with standard error as determined by image analysis in this study.

Computationally restrictive if the spatial coordinates of every possible combination has to be recorded from frame to frame over the period of these videos (30 s). This is an example of what is known as the ‘linear assignment problem’ and is well established in image analysis (Kong et al., 2013; Diem et al., 2015). However, this limit can be overcome by a variety of approaches. We utilized a publicly available software package, the TrackMate plugin for ImageJ, for analysis. In this package, videos are separated into individual frames, and the total number of particles, in this case cells of C. reinhardtii, in each frame are counted by identifying the edges using a Laplacian of Gaussian (LoG) detector, a common approach in image analysis (Leal Taixé et al., 2009; Kalaidzidis, 2009). TrackMate matches particles across multiple frames using the Munkres–Kuhn (aka Hungarian) algorithm which identifies the most likely matches for the particles between frames and is also a well-established method for tracking multiple particles (Kong et al., 2013; Diem et al., 2015).

Visualization Cells and Quantifying Motility

We selected C. reinhardtii cc124, a common lab strain, for our initial experiments as it is the parent line for a number of motility mutants. Cultures were grown for 72 h in standard TAP media at room temperature to a density of 10<sup>7</sup> cells/ml (see Methods). Thirty-second videos of 30 µl aliquots of cultures were acquired and subsequently processed and analyzed in ImageJ with the help of the TrackMate plugin. A representative image slice extracted from one of the videos is shown in Figure 1A. Each frame of the video is automatically separated into different slices and the particles (algal cells) counted (Figure 1B). C. reinhardtii is known to form motile aggregates such as diads and tetrads, which could skew the counting process. However, the software reliably distinguished individual members of this collection of cells (Figure 1C). From these videos, we were able to acquire 3,000–6,000 individual tracks for analysis over a 30-s period (see Supplementary Video 1).

Cells were then tracked frame by frame to determine average speeds (Figure 1D). The average speed of our cc124 populations at 72 h was 40 ± 4.5 µm/s (Figure 1E). These findings are slower than some of those derived from previous studies, which can range from 80 to 200 µm/s (Racey et al., 1981; Marshall, 2009; Engle et al., 2011). However, close inspection of our track speeds confirmed an upper rate of ≈ 93 µm/s, in the range of these previously reported values. A histogram of speed distributions in this population confirmed that >50% of the total tracks for each sample (3,000–6,000 tracks/video) were within the 31- to 60-µm/s range (Figure 1F), consistent with the average speed.

Using Chlamydomonas Mutants to Observe Changes in Motility

A large assortment of motility mutants are available for C. reinhardtii which have helped elucidate the mechanisms of flagellar motility. Unfortunately, the characterization of such mutants is generally limited to relative statements, i.e., “slower” or “extremely slow,” rather than being quantified. In order to confirm the robustness of our tracking approach, we evaluated the swimming speeds of several mutant cell lines of C. reinhardtii. We initially investigated three specific mutant strains: cc1036, a non-motile line, as well as cc2228 and cc3663, both motility-deficient lines. Video analysis confirmed that the total number of tracks for each sample increased in the following order: cc1036, cc2228, cc3663, and finally cc124, respectively (Figures 2A–D). The average speed of the mutant lines ranged from ≈1 µm/s (cc1036) to 20 µm/s (cc3663) based on a minimum of 1,000 tracks/video for each mutant (Figure 2E and Table 1).

Our results establish lines cc1036, cc2228, and cc3663 as examples of 0%, 25%, and 50% motility lines, respectively, when compared to the wild-type cc124 strain. A sample video for cc3663 is provided as Supplementary Video 2 (see Supplementary Material). Closer inspection of the 25% mutant (cc2228) showed that a small fraction of these mutants (<0.01%) were actually able to obtain speeds in the 21- to 40-µm/s category, with a maximal speed of 30 µm/s. The ability to obtain this information provides valuable insight into the stochastic nature of cell populations, but may also be used for identifying unique behaviors and compensatory mutations which may arise. Using this method, we obtained the average cell speeds for 12 different C. reinhardtii mutant lines (Table 1). We note that the cc125 line, which is deficient in phototaxis,
actually moves slightly faster than the cc124 strain (115%, \( p = 0.05 \), as determined by Student’s \( t \) test). In addition to the successful characterization of swimming speeds in mutant lines, this approach appears to have even broader utility as we were also able to characterize the swimming speed of a closely related species, \( C. moewusii \) (48 +/- 6 \( \mu \)m/s).
Characterizing Mixed Populations

Our method was able to measure swimming speeds in populations which converged around a single maximum number of tracks. However, it should also be robust enough to handle mixed populations of varying speeds. In order to evaluate this, we combined equal concentrations of 72-h cultures of cc124 and cc2228, a mutant with 25% motility of wild-type. As seen in Figure 3, the distribution of track speeds for mixed cultures was distinct from either of the cultures for each line individually, with an average speed of 36 ± 2.5 μm/s. These findings underscore the ability of this approach to observe variations within the population.

The Effects of Culture Conditions on Speed

While specific mutations impact swimming speed, it is changing environmental conditions such as nutrient availability and light which influence speed and directionality of motility in the wild type. As previously stated, this particular strain of *C. reinhardtii* is known to be sensitive to nitrogen availability, and we tested the effect of this on swimming speed. We prepared reduced nitrogen TAP by limiting the amount of NH₄Cl added to the media to either 50% or no NH₄Cl. As shown in Figure 4, a fraction of the algae cultured in 50% NH₄Cl TAP (~40%) were observed in the 51- to 75-μm/s range, while in regular TAP only 20% of the cultures reached this range. We propose that this unexpected increase in swimming speed is to support the search for new nitrogen sources in this nutrient-depleted environment. This was in stark contrast to cells cultured in NH₄Cl-free TAP, which significantly reduced motility, not surprising given its importance in flagellar motility and development (Engel et al., 2012). We note that a small fraction of these cultures (<5%) seemed unaffected by the nitrogen availability differences in these media, suggesting some compensatory mechanisms may be at work. Ongoing studies in our lab are further exploring media effects on swimming speeds in this model organism.

Visualizing Phototaxis in cc124

The studies above confirm the utility of this approach to obtain population-level resolution of variations in swimming speed in response to mutation or changing environmental conditions. However, the same method used to determine speed also provides spatial coordinates for each track, allowing us to measure overall changes in the directionality of our samples. In this approach, the coordinates of each track are used to determine where the samples reside within the image. The image space is then divided into eight distinct sectors and populated accordingly. Analysis of our 30-s videos of cc124 in regular TAP media confirmed that the cells were uniformly distributed across the slide, establishing that there is no directionality bias (artifact) in our technique which might impact our study (Figure 5A).

Phototaxis is a commonly observed phenomenon in the cc124 line. Cells will move into the path of light, but also away from the source, presumably to minimize photosynthetic stress. We next sought to exploit this response to see if we could observe population-wide directionality changes utilizing our approach (Harris et al., 2013). Phototaxis was induced by placing a narrow beam of white light (~75,000 W/m²) perpendicular to the focal plane of the slide. As shown in Figure 5B, within 5 min of light exposure, the bulk of the cells in the sample oriented into the path of the light but also into the three sectors furthest from the light source. Surprisingly, there was no increase in swimming speeds within the population in response to this stimulus. These findings confirm the ability of our approach to detect changes in directionality across the window provided by the microscope camera (8.19 mm diagonally across the camera window). While preliminary, this experiment confirms the ability of this approach to observe responses to stimuli within the experimental environment (i.e., the microscope slide). Future experiments will explore the potential for chemotaxis across the surface area of the slide.
DISCUSSION

Automated image analysis provides us with an opportunity to observe and characterize population-level responses, overcoming the limits to statistical resolution and bias associated with manual measurements. In the present study, we have developed a method requiring no custom-designed equipment or software, only a microscope, camera, and the free software package Fiji to analyze the motility of *C. reinhardtii*. Employing this method, we were able to observe differences in swimming speeds between the wild-type strain and several motility mutants, providing quantitative values to these mutants for the first time. In addition to motility mutants, we showed that our approach was sensitive to changes in media composition such as nitrogen availability. As expected, the removal of NH$_4$Cl from TAP seriously compromised motility, but unexpectedly, a 50% reduction in NH$_4$Cl resulted in an increase in the swimming speed of a portion (20%) of the cells. We propose that this rate increase facilitates the search for new nitrogen sources under nitrogen-limited conditions.

When coupled to the microplate-based methods we have already developed for investigating the growth and viability of this microorganism, we are now prepared to thoroughly explore and characterize the impacts of changing environmental conditions and/or mutation on multiple aspects of *Chlamydomonas* biology (Haire et al., 2018). Indeed, our ability to measure swimming speeds in *C. moewusii* strongly supports the utility of this approach for understanding motility in this important genus of over 300 species. While focused on this model unicellular eukaryote, the approaches outlined here should be easily exportable to other genera, potentially providing new insights into microbial ecology, modes of pathogenesis, and other aspects of microbial behavior.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AF performed all phototaxis assays and mutant characterizations including experiments and analysis. TH contributed to phototaxis assays, identified mutant strains to be utilized, and assisted in methods development. KC performed all variable growth media studies and analysis. PS obtained the swimming speeds of *C. moewusii*. MR, CS, SN, and NN-K all participated in the initial development of the method. PR and BR helped simplify the method and validate the results presented herein. AP provided all resources and research oversight for the project.

ACKNOWLEDGMENTS

TH was supported by a departmental graduate student fellowship. AF was supported by the Astronaut Scholarship Foundation. KC, MR, CS, and SN were supported by the NSF Biomath REU.
at the Florida Institute of Technology (NSF ID# 1359341). We thank Professors David G. Lynn (Emory University) and Arijit Mukherjee (University of Central Arkansas) for their comments and insights. Publication of this article was funded in part by the Open Access Subvention Fund and the John H. Evans Library.

REFERENCES

Barsel, S.-E., Wexler, D. E., Lefebvre, P. A., and Fernandez, E. (1988). Genetic analysis of long-flagella mutants of Chlamydomonas reinhardtii. Genetics 118, 633–642.

Bloodgood, R. A., and Salomonsky, N. L. (1989). Use of a novel Chlamydomonas mutant to demonstrate that flagellar glycoprotein movements are necessary for the expression of gliding motility. Cell Motil. Cytoskeleton 13 (1), 1–8. doi: 10.1002/cm.970130102

Dien, K., Magaret, A., Klock, A., Jin, L., Zhu, J., and Corey, I. J. (2015). Image analysis for accurately counting CD4+ and CD8+ T cells in human tissue. Virol. Methods 222, 117–121. doi: 10.1016/j.viromet.2015.06.004

Engel, B. D., Ishikawa, H., Feldman, J. L., Wilson, C. W., Chuang, P.-T., Snedecor, J., et al. (2011). A cell-based screen for inhibitors of flagella-driven motility in Chlamydomonas reveals a novel modulator of ciliary length and retrograde actin flow. Cytoskeleton 68 (3), 188–203. doi: 10.1002/cmt.20504

Engel, B. D., Ishikawa, H., Wemmter, K. A., Geimer, S., Wakabayashi, K. I., Hirono, M., et al. (2012). The role of retrograde intraflagellar transport in flagellar assembly, maintenance, and function. J. Cell Biol. doi: 10.1083/jcb.201206068

Haire, T. C., Bell, C., Cutshaw, K., Swiger, B., Winkelmann, K., and Palmer, A. G. (2018). Robust microplate-based methods for culturing and in vivo phenotypic screening of Chlamydomonas reinhardtii. Front. Plant Sci. 9. doi: 10.3389/fpls.2018.00235

Harris, E. H., Witman, G., and Stern, D. eds. (2013). “Motility and Behavior,” in The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use (Academic Press), 89–117.

Huang, B., Pipergo, G., Ramanis, Z., and Luck, D. J. J. (1981). Radial spokes of Chlamydomonas flagella: genetic analysis of assembly and function. J. Cell Biol. 88 (1).

Huang, B., Ramanis, Z., and Luck, D. J. L. (1982a). Uniflagellar mutants of chlamydomonas: evidence for the role of basal bodies in transmission of positional information. Cell 29, 745–753.

Huang, B., Ramanis, Z., Ducther, S. K., and Luck, D. J. L. (1982b). Suppressor mutations in chlamydomonas reveal a regulatory mechanism for flagellar function. Cell 29 (3), 745–753. doi: 10.1016/0092-8674(82)90436-6

Kalaidzidis, Y. J. (2009). Multiple objects tracking in fluorescence microscopy. J. Math. Biol. 58 (1–2), 57–80. doi: 10.1007/s00285-008-0180-4

Kamiya, R., Kuriimoto, E., and Muto, E. J. (1991). Two types of Chlamydomonas flagellar mutants missing different components of inner-arm dynein. Cell Biol. 112 (3). doi: 10.1083/jcb.112.3.441

Kamiya, R. J. (1988). Mutations at twelve independent loci result in absence of outer dynein arms in Chlamydomonas reinhardtii. Cell Biol. 107 (6 Pt 1), 2233–2258. doi: 10.1083/jcb.107.6.2253

Kong, H., Akakin, H. C., and Sarma, S. E. (2013). A generalized laplacian of gaussian filter for blob detection and its applications. IEEE Trans. Cybern. 43 (6), 1719–1733. doi: 10.1109/TCYB.2012.2228639

Kuchka, M. R., and Jarvik, J. W. J. (1982). Analysis of flagellar size control using a mutant of Chlamydomonas reinhardtii with a variable number of flagella. J. Cell Biol. 92 (1). doi: 10.1083/jcb.92.1.170

Kuchka, M. R., and Jarvik, J. W. (1987). Short-Flagella Mutants of Chlamydomonas reinhardtii. Genetics 115 (4).

Lea Taixé, L., Heydt, M., Rosenhahn, A., and Rosenhahn, B. (2009). Automatic tracking of swimming microorganisms in 4D digital in-line holography data. 2009 Workshop on Motion and Video Computing, WMVC ’09. doi: 10.1109/WMVC.2009.5399244

Luck, D., Pipergo, G., Ramanis, Z., and Huang, B. (1977). Flagellar mutants of Chlamydomonas: Studies of radial spoke-defective strains by dikaryon and revertant analysis. Cell Motil. Cytoskeleton 74 (8), 3456–3460. doi: 10.1037/pnas.74.8.3456

Marshall, W. F. (2009). Marshall, W. F. (2009). Quantitative high-throughput assays for flagella-based motility in Chlamydomonas using plate-well image analysis and transmission correlation spectroscopy. Biomed. Screen. 14 (2), 133–141. doi: 10.1177/1087057108328131

Pröschold, T., Harris, E. H., and Coleman, A. W. (2005). Portrait of a species: Chlamydomonas reinhardtii. Genetics 170, 1601–10. doi: 10.1534/genetics.105.044503

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://frontiersin.figshare.com/collections/Computer-Assisted_Tracking_of_Chlamydomonas_Species/4827747

Copyright © 2020 Folcik, Haire, Cutshaw, Riddle, Shola, Nassani, Rice, Richardson, Shah, Nazamolodini-Kachouie and Palmer. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.