Live imaging of unicellular algae for high-content phenotyping

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ARCADIA

Background

Enabling discovery using image-based comparative approaches

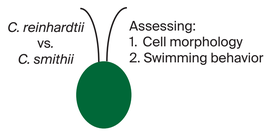
We are working to understand the associations between genotypes and phenotypes across the tree of life. Analyzing variation among interbreeding populations is a powerful tool for dissecting genotype-phenotype relationships.

To build our framework from the bottom up, we're starting by breeding interfertile *Chlamydomonas* species -- *C. reinhardtii* and *C. smithii* -- and performing high-dimensional characterization of many aspects of their biology. Here, we describe multiple phenotypes for each of the two parental species. These phenotypes will serve as a baseline against which we will compare phenotypic and genotypic differences in the progeny of the hybridized species.

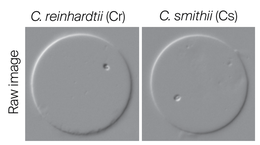
For more, read the full project narrative: **bit.ly/chlamy-parents**

What we are doing:

Experimental Setup



Sample Preparation



Time: ~1 hr to prep samples, ~3 hr for imaging
Check out stamps of different dimensions on Research Microstamps website to find what works for your cells.
Cost: ~$300 per stamp + standard lab supplies

Try out the protocol:

bit.ly_agar-microchambers

How we are doing it:

Image Acquisition

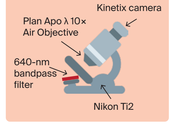


Image Processing & Analysis

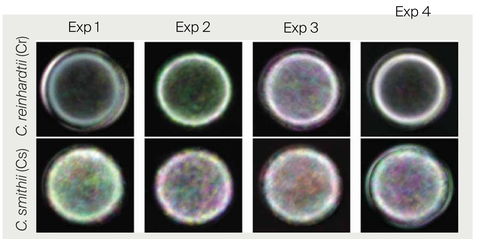
1	2	3	4
Pre-process raw data	Train ilastik	Apply CellProfiler	Parse data with R
Crop, structure folder for batch processing	Generate probability maps from pixel classification	Segment and measure objects	Arrange measurement data into usable tables

Key questions:

1. Can we find a set of traits that quantitatively differ between C. reinhardtii and C. smithii so that we can compare the phenotypes of their progeny back to the parent species?
2. Can we develop simple and scalable methods in order to do high-dimensional imaging?

Findings

Confining cells within microchambers allows for high-throughput imaging

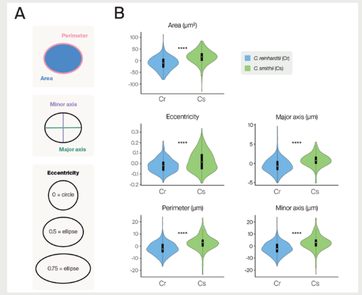


Qualitative comparison of *Chlamydomonas* swimming behavior. Images show the standard deviation projection for cells (n=30) in each experiment (4 total), diameter of circular microchamber is 100 µm.

- To image individual cell behavior, we mounted *Chlamydomonas* cells within 100 µm-diameter agar microchambers (see protocol linked above).
- We imaged 30 cells per species under a 640-nm long-pass filter, using a 10× objective for 3 min at 20 frames per second (total time of acquisition per experiment was ~3 hr).
- Our qualitative assessment (see above) suggested that the species differ in how they explore a confined space with *C. reinhardtii* swimming along the periphery of the microchamber and *C. smithii* exploring the space more uniformly.

Gametes of parent strains differ by morphology and motility phenotypes

Morphology



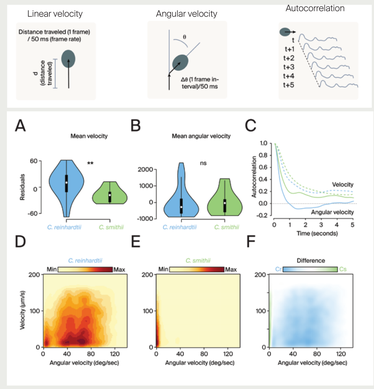
Cell morphology differences between the two species. Violin plots comparing the residuals as a function of species. Asterisks indicate signficant differences for each measure as determined by a Kruskal-Wallis rank sum test and Bonferroni correction. **** p<0.0001

- Gametes of the two species differed significantly across all five morphology measurements, despite experiment-experiment variation.
- C. smithii gametes are ~20% larger (in two-dimensional measurements) and slightly more oblong.

Phenotyping take-home message:

1) Cell morphology differences appeared subtle, but were clearly different between the two species.
2) Temporal variation in velocity parameters is an important feature delineating the patterns of *C. reinhardtii and C. smithii*

Motility



2D motility differences between the two species. Violin plots comparing residuals as a function of species for linear angular velocity when multiple cells were confined within the same microchamber.

- Overall, *C. reinhardtii* display a higher linear velocity, but there was no difference between species in their angular velocity measurements.
- However, the autocorrelation shows a difference in the angular velocity, indicating that *C. reinhardtii* swim in a less predictable way.
- The motility differences were more dramatic when we examined swimming behavior when more than one cell was present.

Immobilizing cells for live imaging allows for visualization of sub-cellular morphology

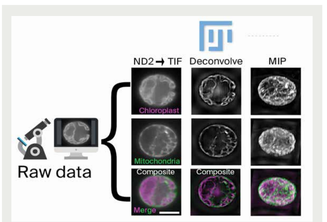
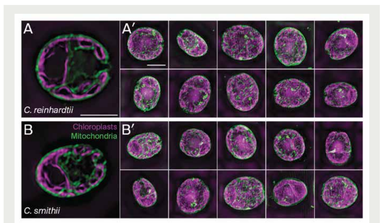


Image processing workflow. Deconvolution, segmentation, and quantification of organelle volumes.



Maximum Intensity Projections (MIPs) after deconvolution. Chloroplast signal (autofluorescence, 640 nm ex) and mitochondria (PK-mito, 561 nm ex). Scale bars 5 µm

- To achieve high-resolution imaging of live *Chlamydomonas*, we adapted a technique for immobilized cells in a low-gelling agarose after staining (Iwai *et al.*, 2018 *The Plant Journal*).
  - Try out the protocol: bit.ly/immobilize-cells
- We imaged cells using a Nikon spinning disk confocal (Yokogawa W1) with a 100×/1.45 NA objective, 2.8× SoRa magnification mode (91 slices, 100-nm step size).
- We used FIJI macros for image processing in batch (see our GitHub associated with the pub, bit.ly_chlamy-parents).
- We will quantify organelle volumes and compare species across life history states (gametes vs. vegetative cells).

Conclusions

- We have densely characterized the phenotypic space and will now assess which combination of phenotypic measures is optimal to differentiate the progeny strains.
- Exploratory differences between the two species are associated with complex patterns of motility that vary over time.
- We hope that these **simple sample preparation** and **open-source image processing tools** enable high-throughput comparative studies across the tree of life.

Next Steps

1. Quantitatively compare organelle morphology between parent species and across life history stages (gametes vs. vegetative cells).
2. Apply these phenotyping approaches to a subset of the progeny strains and compare to the parent species.

I'd appreciate feedback on any of this work, but I'm especially curious about the following:

- Do you have interest in using any of the sample preparation or processing and analysis protocols described here?
- Which methods do you use to segment and quantify images? Any of the ones listed below or others?

Plugins/Packages: ImageJ/FIJI, napari, CellProfiler™ cell image analysis software

Machine Learning: ilastik YOLOv5

Direct Implementation: R, python™

Leave Feedback!

Comment on the pub:

> *Phenotypic differences between interfertile Chlamydomonas species*

Post with #ChlamyParents

bit.ly/chlamy-parents

All other published work: research.arcadiascience.com

A NOTE ON SHARING WITH US!

Part of our mission is to share as much useful research as we can.

If you choose to share a protocol or other useful information with us after viewing this poster, please understand that we may act upon this knowledge and share it when we publish our work. We publish quickly on an independent platform, so this may happen soon after you share, and we cannot wait for you to publish elsewhere.

If you decide to share anyway, yay! That's what science is all about. If your input is useful, we will include you as a contributor to the publication and explain that your role was in providing "Critical Feedback," likely with an additional description of what you shared.

tl,dr — If you're not ready for everyone to know about something, please refrain from sharing it with us.

Contributors (A–Z)

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