

## Project Ariadne: The Effects of Microgravity on the Periodicity of Cytoplasmic Streaming in the Acellular Slime Mold *Physarum polycephalum*

**Abstract:** The proposed project will focus on the exploration of immediate and sustained behavioral changes of the acellular slime mold *Physarum polycephalum* in response to microgravity conditions during a suborbital flight. Specifically, we will be quantitatively and qualitatively observing alteration of cytoplasmic streaming within slime mold plasmodium veins. We will use high frequency and low magnification time-lapse imaging to observe the changes of cytoplasmic streaming velocity and periodicity in an on-board slime mold sample concurrently with a control sample, kept on the ground.

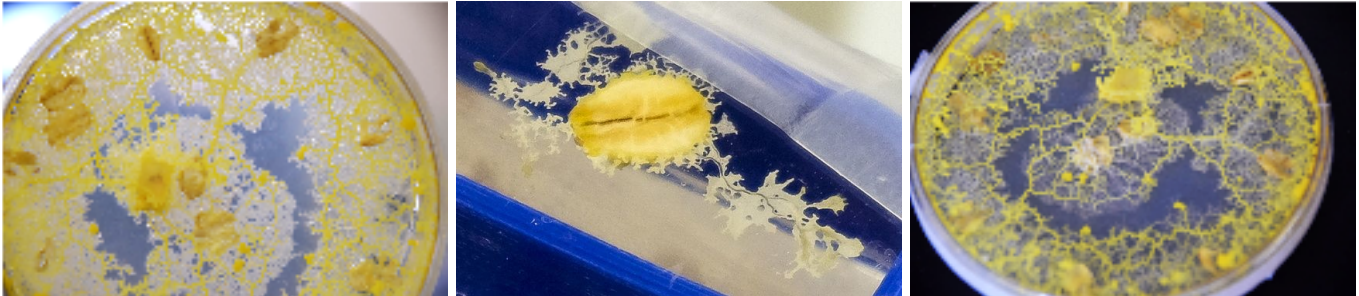


Figure 1: *Physarum polycephalum* slime mold, the proposed experimental subject.

**Motivation:** Cytoplasmic streaming is the movement of cytoplasm to transport organelles, nutrients, and other molecules throughout a cell, and is an important cellular phenomenon present in both animal and plant cells. Due to the acellular slime mold *Physarum polycephalum*'s unusually large cell size and make-up of protoplasm, cytoplasmic streaming can be viewed at relatively low magnification (10x-20x). This makes slime mold an ideal organism for observing cytoplasmic streaming.

In this experiment, we will be investigating how microgravity affects the periodicity of cytoplasmic streaming in *Physarum polycephalum*. The information gathered in this experiment will benefit our understanding of microfluidics and cellular dynamics in space, and can be applied to the investigation of cellular phenomena in other organisms, such as humans. While the physiological implications of cytoplasmic streaming are not well understood, myosin motor movement, the main mechanism behind cytoplasmic streaming, is important to human muscle contraction. Additionally, the velocity and periodicity of cytoplasmic streaming are significant factors in the efficiency of cellular nutrient transport, which is an important consideration for long term human exposure to microgravity.

**Description of Studied Organism:** *Physarum polycephalum* is an acellular slime mold member of the Amoebozoa. The organism is a large, single-cell amoeba, which contains millions of nuclei. The cell contains many internal networks of channels that transport cytoplasm, surrounded by an external protoplasm membrane. This process of cytoplasmic streaming (also referred to as cyclosis, or endoplasmic streaming) is driven by pressure gradients, which are formed by myosin motor molecules moving across actin bundles. The movement of slime mold cytoplasm can be seen at low magnification levels, and velocity and direction changes of the flow can be viewed in real time.

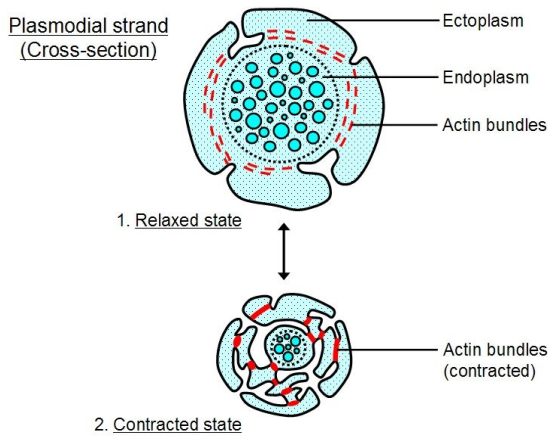
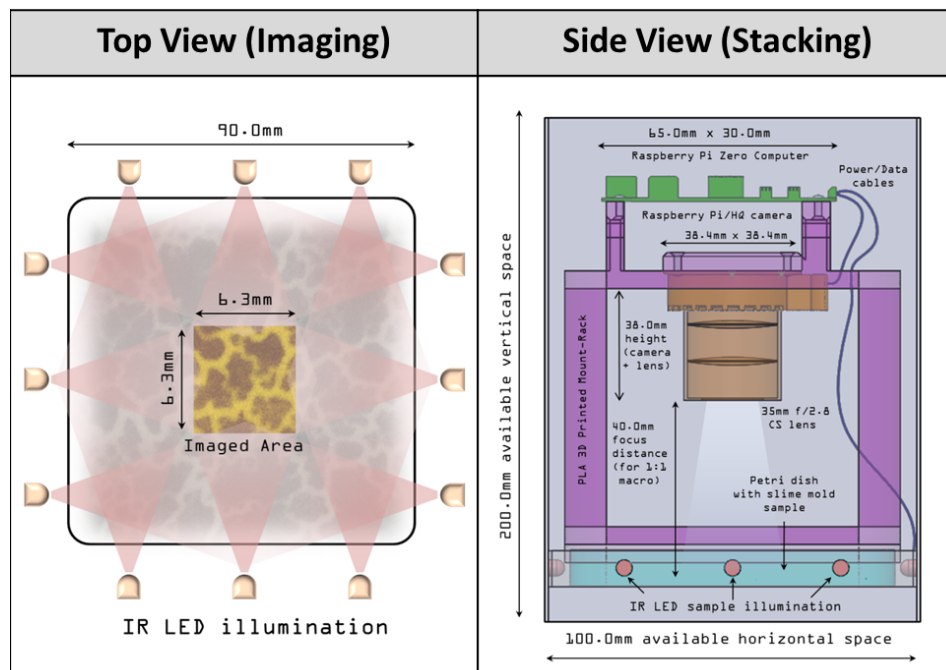


Figure 2 (left): An acellular slime mold's plasmodial vein in both a relaxed and contracted state due to myosin molecule movement across actin filament, the driving force of cytoplasmic streaming.

Figure 3 (right): Visible cytoplasmic content within an established central slime mold artery and outer veins. Cytoplasm can be observed changing velocity and direction, therefore altering the periodicity of overall streaming.

**Hypothesis:** We hypothesize that in the presence of microgravity the velocity of the cytoplasmic streaming in *Physarum polycephalum* will increase, because there would be less hydrostatic pressure experienced during streaming due to the absence of gravity. As a result, the periodicity of streaming would also increase. This hypothesis is supported by the findings of Block et al. 1986, which found an increase in endoplasm streaming velocity in *P. polycephalum* after simulated weightlessness using a clinostat.

**Observation Method:** The on-board slime mold sample will be imaged during and after the flight, while the control sample will be placed in an identical payload assembly and imaged on the ground for the same duration. We will compare (both quantitatively and qualitatively) the differences between cytoplasmic streaming in the ground-based control sample and the on-board sample.



Figures 4-6: The payload design, as viewed from the top, side, and as a whole (shown below).

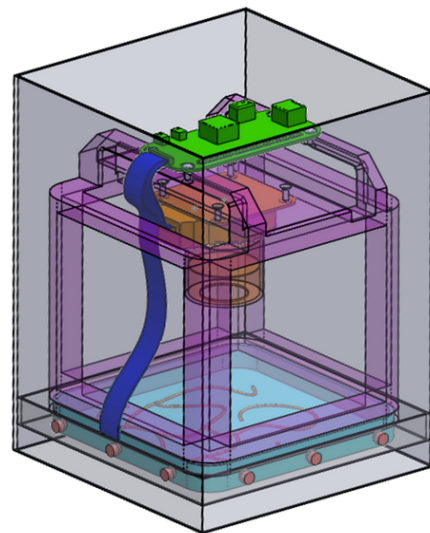
**Payload Design:** We will use the Raspberry Pi HQ camera (which is equipped with a 12MP sensor) attached via a dedicated ribbon cable to a Raspberry Pi Zero to image both the on-board and control slime mold samples. In order to maximize image resolution and quality we will use high frequency time lapse imaging as opposed to video recording.

The sample will be imaged at minimum every second, and because we aim to image cytoplasmic flow, the imaging is set up to provide significant magnification (at least 1:1 macro, resolving features as small as  $1.5\mu\text{m}$ ).

The slime mold samples will be fully contained within sterile Petri dishes and the imaging apparatus will fit inside the Mini Payload provided. The slime mold will be placed on nutrient agar inside a Petri dish in low-light conditions. Slime mold often exhibits strong behavioral reactions to daylight-like light sources; to avoid this issue we will image the slime mold sample under infrared illumination (provided by a strip of IR LEDs, 850nm wavelength).

The power needs of the payload will be satisfied by a 0.9A/5V connection provided by the Mini Payload module. The payload is estimated to need 0.41A/5V for the entire duration of the experiment, and no power is needed when the payload is idle. The details of all payload components are listed in the table below.

### Perspective View (Overview)



Component	Function	Power Requirement	Other Specification
<b>Petri Dish</b>	Sample containment	-	Commercial product
<b>LED strip (12 IR LEDs)</b>	Sample illumination	3.3V, 240mA	Commercial product, 850nm wavelength
<b>Raspberry Pi Zero</b>	Payload CPU/Controller	5V, 100mA (continuous)	Commercial product, 1GHz CPU, 512MB RAM
<b>Raspberry Pi HQ Camera</b>	Sony IMX477 sensor (1/2" format)	5V, 150mA (when capturing)	Commercial product, CS-mount lens, 12MP resolution
<b>CS mount lens</b>	Imaging system lens	-	Commercial product, 35mm focal length, f/1.8 aperture
<b>Plastic scaffolding</b>	Mounting platform	-	In house 3D printed (PLA plastic)
<b>TOTAL Power</b>		2.042W (0.4084A at 5V)	

**Outreach and Social Media Plan:** For two decades, our organization has been building our reputation by participating in numerous STEM outreach events. These events range from single classroom activities at local elementary schools to large public events, including the Physics Open House at UW Madison and the Wisconsin Science Festival (attended by an estimated 35,000 people across the entire state of Wisconsin). In addition to physical outreach, our club also has an established social media presence through Instagram (@westrocketry) and our website (<https://www.westrocketry.com>).

This project will be prominently featured during our outreaches, where we will display a poster and sample payload for the project. We are planning a large scale public announcement of the project at the Wisconsin Science Festival, October 16-17 of 2020. We are also planning a presentation of the project at an Astrobotanical Society meeting this fall.

## KEN SOUZA MEMORIAL STUDENT SPACEFLIGHT RESEARCH PROGRAM – BUDGET (1-2 pages)

Please provide a full budget for your pre-flight, flight, and post-flight research and outreach activities. All hardware development, fabrication, and testing are the responsibility of the proposal team and should be included, as should any associated travel expenses. Indirect costs are allowed; however, it is strongly urged that indirect costs be waived or reduced by the university; the waived indirect costs can be used as matching funds. Any upgrades from the standard Mini Payload offering (i.e., Pad Load) are the responsibility of the proposing institution and should be coordinated in advance with [payloads@blueorigin.com](mailto:payloads@blueorigin.com).

Item	Estimated Cost	Justification
Raspberry Pi Zero Kit, charger, cables, SD card	\$30.00	Payload central computer, drives both imaging and payload illumination
Raspberry Pi HQ Camera, including data cable	\$50.00	Payload imaging subsystem
CS mount lens, 35mm, f/1.8	\$20.00	Lens for imaging subsystem
CS mount extension tube	\$10.00	Macro photography aid
Plastic Petri dishes	\$100.00 (10 pack)	Slime mold containment system
IR LED strip	\$20.00	Slime mold sample illumination
PLA filament (3D printing)	\$50.00 (two spools)	Payload structural subsystem
Slime mold plasmodium culture	\$20.00	Initial slime mold specimen
DIRECT COSTS SUBTOTAL	\$300.00 (per one payload)	
INDIRECT COSTS SUBTOTAL	\$0.00	All indirect costs will be waived by project sponsoring organizations (Madison West High School and UW Madison)

TOTAL COSTS	\$600.00 (two payloads will be needed, the on-board payload and the control ground-based payload)	
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Proposals with a total budget that exceeds the \$1,000 award are encouraged to match award funds with funds from their Institution or other sources. Match can be in the form of either cash or in-kind, including waived indirect costs, student stipends, hardware loans, computer time, or fundraising campaigns.

Cost Sharing Source & Type	Estimated Value	Justification
Workshop space	\$200/month, fully waived	Payload construction
3D Printers Access	No cost, permission granted	Payload structural subsystem production
Lab access at UW Botany Dept. / Gilroy Lab	Cost unknown, provided by UW Madison, all costs waived	Payload preparation, cultivation and general storage for payload imaging
Classroom space	Cost unknown, provided by UW Madison, all costs waived	Documentation preparation, team meetings and training
Online presence	\$16/month, fully sponsored by parents and community	Website and online collaboration tools
Teleconferencing equipment access and use, including connection costs	\$300/hour, provided by UW Madison, all costs fully waived	Teleconferencing, online collaboration, as necessary
Outreach activities, poster printing, display construction	No cost, all outreach activities are fully sponsored by parents and community	Public presentation of the project, scientific outreach and payload demonstrations
TOTAL	\$0.00, all resource sharing costs are fully waived by sponsoring/supporting organizations	

## KEN SOUZA MEMORIAL STUDENT SPACEFLIGHT RESEARCH PROGRAM – SCHEDULE (1-2 pages)

Please outline your expected schedule for pre-flight, flight, and post-flight activities. Include both research and outreach elements. Proposals should assume the following key milestones:

Proposals Due	June 3, 2020
Winner Officially Announced	ASGSR Conference (Nov. 4-7, 2020)
Flight Contract Signed	Launch minus 6 months (L-6 months)
Preliminary Payload Data Package	L-5 months
Final Payload Data Package	L-3 months
Hardware Delivered for Flight	L-2 weeks
Flight (L-0)	2021
Presentation at ASGSR 2020	Fall 2021
Summary Report to ASGSR	L+3 months

Proposed schedule\*:

<b>2020</b>	June 3	Proposal submitted
	August 1 - August 31	Project poster and initial documentation - outreach team, online collaboration
	August 1 - August 31	Imaging system assembly, programming - imaging team, online collaboration
	August 1 - August 31	Payload prototype design and construction - payload team, online collaboration
	September 1 - October 15	Review of prototype phase, preparation for public introduction of the project at Wisconsin Science Festival
	October 16 - October 17	Project introduction at WI Science Festival

	October 21	Project presentation at Astrobotanical Society
	October 22	Lab work begins, payload samples cultivation
	November 4-7	ASGSR Conference, KSG Announcement
	November 10	Payload expected fully functional, imaging tests and full project documentation begin
<b>2021</b>	January 15	Preliminary Payload Data Package submitted
	January 15 - March 15	Payload testing/improvements continue
	January 15 - March 15	Public outreach events continue (including Physics Open House / Wonder of Physics at UW Madison)
	March 15	Final Payload Data Package submitted
	March 15 - June 1	Payload testing/improvements continue
	March 15 June 1	Public outreach events continue
	June 1	Payload delivered for flight
	June 15	Estimated launch date, payload recovery
	June 15 - June 30	Payload data analysis
	July 1st	Flight samples of slime mold offered to other research groups
	July 1 - July 31	Preparation of presentation for ASGSR 2021
	August 1 - August 31	Updates of outreach materials
	September	Summary Report Submitted
	October	Project results presented at Wi Sci Fest 2021
	Fall	Presentation of project results at ASGSR conference
	December	Official project closure, photo-book distributed

\*Because of the strong interest of students in the project, time demands for development of a biological payload, intrinsic value of the proposed payload and thematic alignment with our other astrobotany projects, the project schedule will start this summer with the full understanding that only one of the submitted proposals will be chosen by the KSG committee.



**Appendix****References**

Block, I., Briegleb, W., Sobick, V. and Wohlfarth-Bottermann, K. (1986). Confirmation of gravisensitivity in the slime mold *Physarum polycephalum* under near weightlessness. *Advances in Space Research*, 6(12), pp.143-150.

Block, I., Wolke, A., & Briegleb, W. (1994). Gravitational response of the slime mold *Physarum*. *Advances in Space Research*, 14(8), 21-34. [https://doi.org/10.1016/0273-1177\(94\)90382-4](https://doi.org/10.1016/0273-1177(94)90382-4)

Koritsas, E., Sidiropoulos, E., & Evangelides, C. (2018). Optimization of branched water distribution systems by means of a *Physarum*—Inspired algorithm. *Proceedings*, 2(11), 598. <https://doi.org/10.3390/proceedings2110598>

Reid, C. R., Latty, T., Dussutour, A., & Beekman, M. (2012). Slime mold uses an externalized spatial "memory" to navigate in complex environments. *Proceedings of the National Academy of Sciences*, 109(43), 17490-17494. <https://doi.org/10.1073/pnas.1215037109>

**Figure Citations**

Fig. 2. (2018) *Plasmodial Strand*, [digital diagram]. Retrieved May 26, 2020 from Cronodon: [https://cronodon.com/BioTech/Plasmodium\\_SM.html](https://cronodon.com/BioTech/Plasmodium_SM.html)

Fig 3. (2013) *Cytoplasmic Streaming in Slime Mould Physarum*, [digital video]. Retrieved May 26, 2020 from YouTube: <https://www.youtube.com/watch?v=UKSGAHafWis>