The dynamics of *Physarum polycephalum* fungitaxis and photoavoidance.

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Authorization to Submit Dissertation

This dissertation of Leo Epstein, submitted for the degree of Master of Science with a Major in Bioinformatics and Computational Biology and titled "The dynamics of *Physarum polycephalum* fungitaxis and photoavoidance.," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

In my thesis I have tried to answer the following questions: What effects do different patterns and amounts of light exposure have on *Physarum* fungitaxis and morphogenesis? Are the dynamics of *Physarum* and yeast interaction modulated by light? Can we characterize the *Physarum* light response and its interactions with a red yeast such that we can input a sequence of light into *Physarum*'s environment such that we can increase or decrease the rate at which it consumes a red yeast (we have not genotyped this micro-organism). *Physarum* morphogenesis and fungitaxis(how it interacts with a red yeast) were measured using several week-long image sequences taken with flatbed scanners. These images were segmented using the semi-supervised iterative pipeline of U-Net and Ilastik. These semantic (as in we defined elements of the image that should be labelled by the network) segmentations were used to measure the mass and morphology of yeast, oats, and *Physarum* in all images. With these measurements, we analyzed the interactions shared between *Physarum* and red yeast. We find that these interactions occur at specific spatial scales. Running our data through the FNN-LSTM autoencoder help us to better understand the dynamics of these interactions. We find that *Physarum* and yeast interactions are succesional. *Physarum* maintains yeast as a periodic food source by depositing a nutritive slime trail after it consumes most of the yeast. With these observations we moved on to the other component of our research program, how *Physarum* morphogenesis is influenced by light exposure. To answer this question we trained a triplet loss embedding (a supervised clustering algorithm that works on user defined/semantic labels). We were unable to generate satisfactory clusters with this embeddinga/clustering algorithm. Individual plasmodia clustered with each other in temporally coherent sequences within the latent space. Instead of constructing a latent space with which we could understand photomorphogenesis and photomovement we found a pathological feature of the Triplet embedding algorithm. With this failure in mind we tried to construct a morphospace of *Physarum* morphogenesis. With this morphometric approach we were unable to find evidence that photoexposure itself shapes *Physarum* morphogenesis over the course of a week. This observation in some ways contradicts the existing literature in which *Physarum* avoids

blue light(Whiting *et al.*, 2014) and the peristaltic pumping that drives *Physarum* movement is disrupted by light exposure(Block and Wohlfarth-Bottermann, 1981). It is possible that *Physarum* habituates to the regime of light exposure and loses sensitivity during the course of an experiment. To investigate this hypothesis, we propose new experiments to better understand the temporal dynamics of photoavoidance (how *Physarum* avoids a particular region of light) in contrast to photomorphogenesis where its whole environment is illuminated. With these new observations, we may be able to better understand *Physarum* photoavoidance and its relationship with fungitaxis. We may also be able to characterize a decay in the *Physarum* photoavoidance response that would in itself be an important result.

Acknowledgements

I would like to thank My family, Jerry Garcia, Bad Brains, Gucci Mane, Greg Nuckols, White2tea and last but not least the Preibisch lab. They have allowed me to complete this thesis. I would like to thank Zeth Dubois for his helping me a great deal with the work described in the thesis.

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CHAPTER 1

Introduction

In the research described here, I attempt to characterize the fungitaxis and photomorphogenesis behaviors of the acellular slime mold *Physarum polycephalum*. We assumed that these behaviours would be manifest through morphogenesis comparably across *Physarum* of differing nutritional states and stress loads. If the *Physarum* growth response to light differs between plasmodia (a single *Physarum* is called a plasmodia) of differing hunger levels, then we unlikely to find a robust link between light exposure regimes and programs of morphogenesis. On the other hand, fungitaxis may be directly observed in the images. Quantifying how fungitaxis and photoavoidance behaviours manifest across different nutritional-ecological and light-exposure regimes might reveal some aspect of the biophysical computation performed by *Physarum* to plan future growth. To measure these behaviors and their putative interactions, I constructed an experimental and analysis pipeline. First images were semantically segmented using U-Net (Ronneberger *et al*., 2015). Information was extracted from the U-Net semantic segmentations using the Python image analysis stack (Numpy, Skimage, and scipy) and ImageJ scripting. With this analytical stack I attempted to characterize *Physarum*'s response to fungi (fungitaxis) as well as light (photomorphogenesis). We attempted to correlate the light exposure regime (e.g portion of the time light is turned on) to *Physarum* morphogenesis. Others have shown that light exposure modulates the frequency of the peristaltic contractions that drive *Physarum* morphogenesis over shorter time scales(Block and Wohlfarth-Bottermann, 1981),(Durham, 1976). We reason that if these changes in peristalsis persist for days and weeks, they will manifest themselves as different morphogenic programs because peristaltic pumping drives *Physarum* morphogenesis at fine temporal scales. Previously, changes in peristalsis have been observed across hours and minutes.(Block and Wohlfarth-Bottermann, 1981),(Durham, 1976)

Across our experiments we wanted to test the hypothesis that *Physarum* translates a sequence of light into a morphogenic program. To this end an experimental appara-

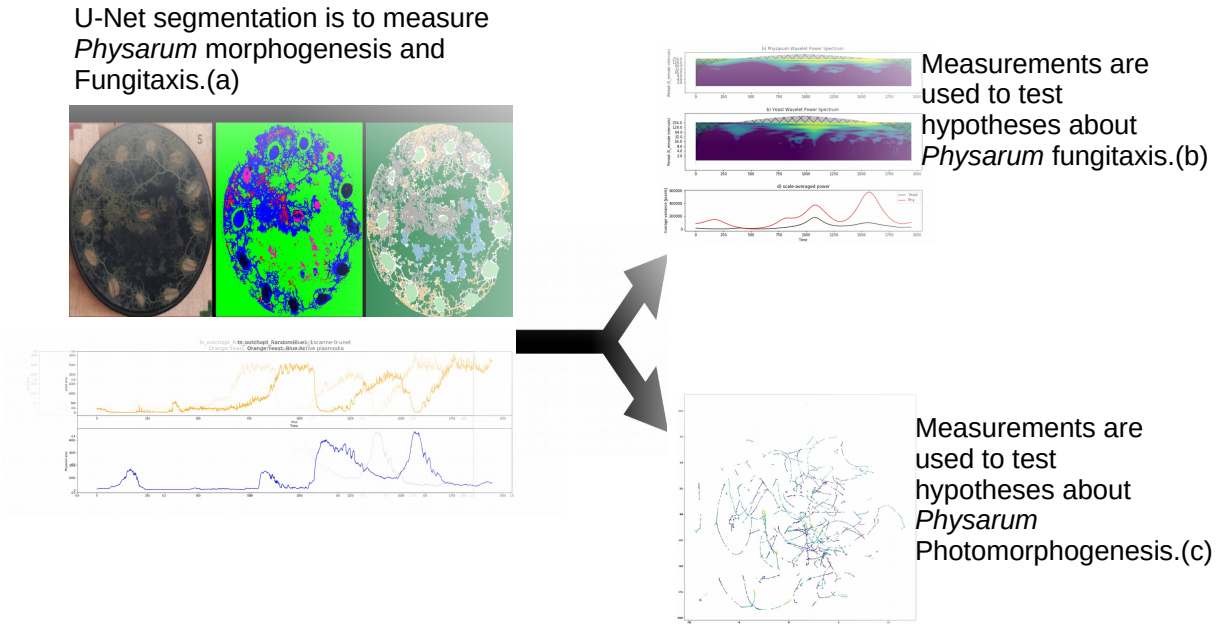

FIGURE 1.1: A graphical abstract with cartoons of segmentation (a), measurement of *Physarum* yeast ecological interactions (b) and a triplet loss embedding space of *Physarum* morphogenesis (c). morphogenesis

tus was built to expose *Physarum* to light and measure any response that this exposure may elicit. An LED installed atop the *Physarum* enclosure, is randomly turned on or off for five minute intervals. Images are also acquired at five minute intervals. By choosing low or high threshold values for this light switch we create high or low light regimes for *Physarum*. Across these regimes we may measure light exposure as a sequence of binary elements (for example: On, Off, On, On, On). Each of these elements corresponds to an image of *Physarum* in its experimental enclosure. This random approach to light exposure was chosen over others for several reasons. It was believed that any correlation discovered between *Physarum* morphogenesis and a random light sequence would be more easily adapted to any control algorithm that we ended up developing. It was believed that random light would allow us to get a fuller accounting of *Physarum* morphogenesis. In any future experiments it would be interesting to study the effects that a periodic light stimulus might have on *Physarum* morphogenesis. Illumination and darkness could be cycled between over a period of hours or days. Dynamics of plasmodial photomorphogenesis could be quantified across time in relationship to dark-light and light-dark transitions. With our random light program it is less obvious as to how easily tested hypotheses about light exposures over a period of times correlation with *Physarum* morphogenesis might be made. With these images and light measurements we tried to correlate random light exposure sequences to *Physarum* morphogenesis. Ultimately we were unable to identify any consistent *Physarum* morphogenic light response. In each of the plates in which *Physarum* was grown, a ring of oats was placed along the margin; an oat was placed at each hour notch on the face of a clock. The oat at 6 o'clock was replaced with an oat colonized by a red yeast. *Physarum* was placed in the center. While oats are a food source for both *Physarum* and the red yeast, there is no reason to think they will inhibit fungitaxis or confound our measurements of *Physarum* yeast interactions. The oats are small in size and do not isolate the organisms from each other. Like whale fall in the deep ocean, oats support a web of interspecies ecological interactions. It is also the case that our spatiotemporal measurements of *Physarum* yeast interactions may be carried out in such a way that regions around oats are not measured. With these measurements we can assess

the putative effects of light exposure on *Physarum* red yeast interactions. Varying patterns and exposure times of blue light may effect patterns of *Physarum* morphogenesis, these changes in morphogenesis may have downstream effects on patterns of slime sheath deposition and, yeast predation. Across experiments, the light exposure regime was varied (as in light exposure per interval was more or less likely), these experiments and their timelapse/timeseries of corresponding image light status pairs were processed through a computational analysis pipeline (described in greater detail below). With the products of this pipeline we pursued the ambitious goal of characterizing *Physarum* photomorphogogenesis and fungitaxis with the hope that we could test hypotheses about the relationship between photomorphogenesis and *Physarum*-yeast ecology using neural networks. Across our attempts to characterize *Physarum*-yeast ecology and its light response we drew heavily on literature that described *Physarum* behavioral ecology, photomorphogenesis / photo chemistry, as well as the literature on neural networks. Experiments were undertaken and data collected with the overarching goal of creating a training corpus to train these neural networks. A further (very ambitious!) ambition was to create a means to influence *Physarum* fungitaxis with some sort of PID controller conditioned on a photomorphogenesis embedding space. Obviously this goal was not met, but mentioning this should provide some context as to why we ran so many experiments with a particular configuration of *Physarum*, red yeast, and oats. This also explains the bifurcated structure of the thesis with one analysis section focused on the analysis of *Physarum* red yeast interactions and another detailing our attempts to characterize *Physarum* light response. If we were to have succeeded we would have demonstrated that we had created a robust and meaningful embedding of *Physarum* photomorphogenesis. Germane background to these two analysis sections is provide in an overview of *Physarum* biology as well as the computational methods we used to processs and analyze the image data we generated. The introduction continues with an overview of the neural networks we attempt to characterize *Physarum* photomorphogenesis and red yeast interactions. Neural networks have recently become popular for morphometric analysis. Networks have been used to characterize how the fine grain structure of neurons correlate with broader neuron function and how patterns of

spatial gene expression exist in the murine brain (which sorts of genes have the same spatial patterns of expression). (French), (Schubert *et al.*, 2019). These networks learn to semantically cluster the data-label pairs they are trained with. In French et al. images of gene expression in the murine brain are clustered with genes with similar spatial patterns of expression clustered together in the embedding space. This clustering gives us some idea of the relationships that the gene labels share as per their spatial expression in the murine brain. In Schubert et al. Electron microscopy (EM) cuboids are embedded based on the type of neuron they belong too. The clusters generated by the embedding neatly separate EM cuboid subsections based upon the functional type of neuron (the label class) they belong to. With this sort of metric embedding technique the authors are able to demonstrate that neuronal morphologies differ across scales (EM cuboids up to whole neurons). I hoped to use similar methods to correlate specific regimes of illumination to different patterns of *Physarum* morphogenesis. As *Physarum* migrates across its environment, it changes shape as it looks for and harvests food(Oettmeier *et al.*, 2018). As certain types of EM cuboids are associated with kinds of neurons are certain *Physarum* morphogenic motifs associated with a particular recent light regimes? How might different light regimes correlate with the frequency and timing of morphological transitions? I wanted to identify (with triplet loss in addition to other methods) biological processes that may be involved with these transitions. The ways these morphologies (encoded as images) might cluster in a photomorphogenesis space (labels from our measurements of light exposure) would shed light on the processes that drive the dynamics of *Physarum* morphogenesis.(Oettmeier *et al.*, 2018), (Ray *et al.*, 2019), (Alim, 2018) We also use Neural networks to characterize the interactions that *Physarum* and a red yeast share. Using the FNN-LSTM architecture developed by William Gilpin we try to learn the strange attrator dynamics of *Physarum* - yeast predator prey interactions (Gilpin, 2020). With the aforementioned neural networks we can test hypotheses about complex biological questions. With the triplet loss clustering we can characterize how sequences of light exposure (across time) correlate with *Physarum* morphogenesis. Training the the FNN-LSTM we can get a sense of the spatiotemporal dynamics of *Physarum* - yeast interactions. To our knowledge we are

the first to characterize *Physarum* fungitaxis and attempt to characterize *Physarum* morphogenesis over a longer (over the coures of a week) time horizon. This has implications that may be better understood after reading a summary of the research done on *Physarum* morphogenesis and ecology as described below. The Thesis is structured in five sections; the biology of *Physarum* morphogenesis is described, an overview of the neural networks we used as well as other implemented computational techniques is given, experimental design is described, and our results are presented.

CHAPTER 2

Physarum Biology

2.1 *physarum* fluid flow

Physarum, in contrast to neural organisms, does not have a specific region from which control emanates. (Alim, 2018) No one has identified the biochemical processes/biological structures that drive *Physarum*'s interaction with its environment as the brain and nervous system do in neural organisms, we do not know how *Physarum* modifies the dynamics of fluid flow within its vasculature. Karen Alim, in her foundational paper, shows that, across short time scales (tens of minutes), *Physarum* morphogenesis is shaped by the fluid flow within its vascular network as bioelectric signals that propogate through our nervous systemm shape our movement on the scale of tens of minutes.(Alim, 2018) The approximate orientation of the fluid flow in the endoplasm (approximates the cytoplasm of a bacteria) is organized in a peristaltic wave. This flow directs growth in *Physarum*. (Alim, 2018) In the case of *Physarum* a peristaltic wave is generated in a tube of actin, containing endoplasm that undergoes radially symmetric contraction and expansion; moving fluid down the actin lined tube. As the dynamics of cytoplasm flow change *Physarum* morphology changes in kind. This is a result of differential diffusion of nutrients and organelles contained in the endoplasm (These nutrients and organelles can be used to create more actin lined tubules). Peristaltic contractions are organized by biochemical signals present in the endoplasm. The signal(s) modulate(s) the rate of contraction across the vascular network. Others have demonstrated that the vasculature can be arranged in such a way that a logical statement can be evaluated from information contained within the cytosol. (Whiting *et al.*, 2014), (Tsuda *et al.*, 2004). As the cytosol flows through the vasculature it gives rise to a wide range of computations as documented by Whiting et al. (Whiting *et al.*, 2014). In the aforementioned paper, plasmodial vasculature was grown so its constituent tubules were organized into a logic gate. Each node of the gate was grown on an oat on agar atop a heating element. Heat is

an attractive stimulus to *Physarum*. By heating certain nodes in these vascular circuits the authors were able to approximate OR, AND, NOT, XOR, Half Adder, and Full Adder gates. By using biosensors, the oscillatory electric activity (actin contractions) of the connected protoplasmic tubes was measured and decoded. The *Physarum* OR and NOT gates were able to provide an accurate output 90% of the time. The other gates provided a correct output 60-70% of the time. As the vascular extent of the gates grew their accuracy decreased. This paper demonstrates that fluid flows of the *Physarum* protoplasm can approximate logic gates. This provides evidence of *Physarum*'s computational capacities derived from its fluid flow. The physical mechanisms of how *Physarum* uses fluid flow to grow and make the complex decisions documented by Whiting et al. can be measured as they have been done by Alim. The biochemical processes that shape endoplasmic flow and, over longer periods, morphogenesis are unknown. Ray et al document how these unknown biochemicals are transferred across the plasmodia as *Physarum* makes a choice.

2.2 *physarum* TAXIS

Physarum taxis Building on Dr. Alims' work, others have measured the peristaltic contractions of *Physarum*'s vasculature and used these observations to approximate information flow in the cytosol. Ray et al. hypothesize that some signal in the cytoplasm elicits a morphogenic response/ performs a computation (Ray *et al.*, 2019). By measuring peristaltic tubule contractions, Ray et al identify how *Physarum* organizes fluid flow so that it can make a binary nutritional choice.(Ray *et al.*, 2019) In experiments by Ray et al, a plasmodial tubule is grown between two nutritionally distinct food sources. Ray et al hypothesize that a solute contained within *Physarum* cytoplasm contains some sort of signal that *Physarum* uses to coordinate contraction across its vasculature. The dynamics of information transfer across the tubule are measured by calculating the rates of contraction across the length of the tube over time. Rates of contraction across the tubule are lower nearer to the higher quality food source and higher near to the low-quality food source. Ray et al conclude that a signal is propagated from the end of the tube with a low-quality food source to the end of the

tube with a high-quality food source.(Ray *et al.*, 2019) The fluid flow carries a signal that over some time horizon organizes *Physarum* taxis to the food source. *Physarum* distributes biological signals within its body to direct fluid flow. *Physarum* may also leave behind signals in the environment as discussed in the next paragraph. Work in Dr. Dussotours group has also shown that *Physarum* can perform embodied computation. *Physarum* can change its environment to more efficiently avoid negative stimuli and forage for food (Boussard *et al.*, 2019).*Physarum*, while migrating, deposits a slime sheath.(Reid *et al.*, 2012) This slime sheath, like many other cues in the environment (incline, light, heat, nutrition source, and micro-organisms) are processed by *Physarum* so that it can avoid them or move toward them. These stimuli direct *Physarum* migration and more generally morphogenesis (Vogel *et al.*, 2016). In Reid et al. (Reid *et al.*, 2012) *Physarum* were placed in an agar plate in which a food source was blocked by an obstacle (a plastic block around a portion of an oat). In one condition the agar was coated with slime-sheath, in the other condition the agar was untreated. Across these two conditions, the time taken to reach the food source was measured. The *Physarum* placed on a plate coated in slime-sheath took longer to navigate to the food than its counterpart in the untreated environment, in a coated environment the slime-sheath was a useless signal. On the other hand by avoiding the slime-sheath, *Physarum* was able to navigate to food more efficiently. By modifying its environment *Physarum* can better undertake complex tasks such as foraging. This observation clearly shows that biochemical signals in the environment interact with fluid flow to shape *Physarum* morphogenesis. While the aforementioned papers described the phenomenology of *Physarum* morphogenesis other papers have suggestions as to the identities of the biological processes that organize *Physarum* morphogenesis. A paper describing the ultrastructure (electron microscopy images) of the *Physarum* plasmodia suggests some of the possible identities of the biological signals within the cytoplasmic flow that shape morphogenesis and aid in decision making as per Ray et al. The EM data depicts clusters of organelles inside the endoplasm. (Rhea, 1966) These organelles include the endoplasmic reticulum and mitochondria. In one micrograph, mitochondria are clustered at the edge of an extending plasmodial pseudopod (tip of a *Physarum* tubule). Perhaps these mitochondria are involved in shaping actin dynamics that

drive *Physarum* morphogenesis (laying new railroad track for the plasmodial express). How are the mitochondria supplied with energy? Some portion of the dynamics that drive *Physarum* morphogenic behavior are probably visible in the succession of morphologies that *Physarum* transitions through as it interacts with its environment and consumes and spends nutrients. These mitochondrial clusters might be explained by morphological features of the pseudopod or might be associated with regions of low flow. In light of (Ray *et al.*, 2019), a story could be told where mitochondria are being diffused from a region of low nutrition to a region with higher nutrition via fluid flow as per (Alim, 2018). As *Physarum* migrates across its environment it is lead by a region of actin synthesis/with less vascular structure; this is called a growth front. A stationary or vascularized plasmodia might have a more complex vascular morphology, whereas a migratory plasmodia has a less articulated vascular structure with a more promminent growth front. These differences in flow may also correlate with compositional differences in the cytoplasmic solute. As with multiagent swarms, different *Physarum* morphotypes (migratory and sedentary) have different multi-agent interaction types (Carrillo-Zapata *et al.*, 2019). While unsimilar at first glance multiagent swarms and *Physarum* share some similarities. Summary statistics that describe the change in morphology over time are used to gain insight into the behaviour of the emergent interactions between agents in the swarm. These sorts of morphological summary statistics allow the swarm researchers to characterize the ways in which the different parameters they plug into the programs organize swarm growth. Like the aforementioned swarm researchers, we think of light exposure regime (how much light has the *Physarum* been exposed to in the last hour) as a parameter. We would like to characterize how different amounts and types(where type is the random number generator that decides whether light is turned on or off) of light exposure shape *Physarum* growth over time. We think of *Physarum* morphogenesis as a program that has light arguement that can be modified; we attempt to characterize how changing this light variable affects the output of the *Physarum* morphogenic program. Varying the amount of light exposure over the course of an hour in our experimental system might be compared to changing the rate at which signals diffuse in a multiagent swarm (the rate at which agents are able to communi-

cate with each other). By modifying how signals spread in a multiagent swarm you substantially alter their morphogenic behaviour. In *Physarum* these different types of agents could be thought of as regions of the plasmodia in which the cytosol has varying concentrations of organelle solute in the cytoplasm as per Rhea et al. Regions with less mitochondria may be less dynamic than their mitochondria rich neighbours. Mitochondria in this case could be thought of as sources of energy; as different portions of the *Physarum* vein network interact with each other concentrations of organelles and nutrients (including mitochondria) are moved around the plasmodia. This differential diffusion of organelles and nutrients organizes morphogenesis in *Physarum* as per Ray et al(Ray *et al.*, 2019). It would be very exciting if this sort of morphospace schema could be shown to be ueful in describing morphogenesis and the putative (as described above) processes that drive it. It would suggest that the rationale of multiagent swarms could be extended to other biological systems. How do diffuse biochemical and biophysical gradients produce a complex suite of behaviors in *Physarum*? Researchers of swarm/collective intelligence are interested in *Physarum*'s ability to meet environmental challenges without any visible centralized computation. (Carrillo-Zapata *et al.*, 2019) These similarities might suggest that by modifying biological substrates of computation with a stimulus such as light you might be able to consistently control some facet of *Physarum* morphogensis (in the context of multi-agent swarms you would change parameters governing growth). This of course, would not be true if light effected *Physarum* morphogenesis by killing off mitochondria; past a certain point the stimulus would have a different effect on *Physarum* morphogenesis. We hypothesize that light exposure per hour is some sort of paramater that can tune aspects of the program of *Physarum* morphogenesis with out changing it.

2.3 *physarum* INTERACTION WITH MICROORGANISMS

Physarum interaction with microorganisms With regards to the behavioral ecology of *Physarum*, we test hypotheses about the ways in which *Physarum* may interact with other micro-organisms. We hypothesize that a red yeast eats the *Physarum* slime

sheath. In a more natural ecology, this sort of public good dynamic is common. Bacteria (In our case a protist *Physarum*) produce metabolites that invite other microorganisms to grow near them. (Nadell *et al.*, 2016),(Nadell *et al.*, 2008) *Physarum* have been documented living in and under rotting logs; rich ecosystems; hotbeds of public good productio and consumption. Interrogating how *Physarum* interacts with a red yeast may help us contextualize many observations made about (fungi)taxis behavior in the lab. Working with image data we may characterize the spatiotemporal dynamics of an interspecies interaction. In our analysis we borrow heavily from the lexicon and methodology of ecology. Our experimental system is fertile for ecologists who would like to understand patchy unmixed interactions more generally. In many cases the dynamics of studied ecological systems work themselves out over times scales on the order of years, or occur on the spatial scale of biofilms. *Physarum* and yeast provide a toy ecology that has dynamics that work themselves out over the course of a week or so and can be observed without a microscope. It is also the case that *Physarum* and the order *Mxyomycetes* play an important role in the ecosystems that they inhabit (as microvores) yet observations about their behavioral ecology are few and far between.(Ing, 1994) Even in the existing *Physarum* fungivory literature, the spatiotemporal dynamics of *Physarum* – microorganism interactions have not been addressed in great detail. Fungivorous and bacterivorous behavior has been observed across the class Mycetozoa (including *Physarum*). (Chapman and Coote, 1983), (Cohen, 1939). Chapman and Cohen observe that *Physarum* and other acellular slime molds such as *Badmania* grow much more vigorously when they are placed in an environment with yeast and other microorganisms. Others have observed that plasmodia growing in a two-member culture survive longer than they otherwise would and demonstrate a deepened behavioral repertoire (more dynamic growth).(Cohen, 1939), (Chapman and Coote, 1983) Cohen et al hypothesize that *Physarum* grown in a two-member culture, consumes the yeast while the yeast breaks down the complex carbohydrates locked up in the oats. In the context of our experiments, *Physarum* may either utilize a nutrient-poor ring of oats, or invest energy in foraging for yeast. While *Physarum* certaintly consumes a red yeast, the time and tempo of this behaviour has not been characterized. *Physarum* and yeast could have a predator prey relationship,

or the deposited slime sheath could introduce a succesional dynamic into this bipartite ecological assemblage. By characterizing the interactions between *Physarum* and a red yeast we may come to a better understanding of the dynamics of their relationship. This would contribute to the sparse but interesting literature on *Physarum*'s behavioral ecology. In addition to predator prey interactions with micro-organisms *Physarum* has been shown to enter into symbiotic relationships with other protists. Waldo Lazo has shown that, across many different *Myxomycete* (the class of organisms in which *Physarum* belongs) – green algae species pairs, a symbiosis can arise. Lazo quantified symbiosis by measuring the color of the *Myxomycete* across time. As algae grows in the endoplasm the *Myxomycete* turns a shade of green. (Lazo, 1961) Gastrich et al. observed that after several days albino *Physarum* plasmodia would take on a green color. (GASTRICH and Anderson, 2002) Gastrich et al. have shown that growing an albino variant of *Physarum polycephalum* in association with the photosynthetic protist, *Chlorella pyrenoidosa* increases the longevity of *Physarum* from 5 to 10 days to up to a month. Like Lazo they assess symbiosis by measuring the greeness of the albino *Physarum*. They find it takes approximately one week for the plasmodia to turn green. Gastritch et al. use a TEM to perform ultra-structure analysis. They find the *Chlorella* within membrane compartments of *Physarum polycephalum*. The authors hypothesize that *Chlorella* can provide *Physarum* with nutrients it produced from Photosynthesis. *Chlorella*'s green pigment may protect the albino slime mold from harmful illumination. *Physarum* as well as other Mycetozoa, may form intimate beneficial relationships with many different types of microorganisms. Others have not attempted to quantify the spatiotemporal dynamics of these sustained and unique types of interactions. As we study the interactions between these two different species (*Physarum* and a red yeast) we mainly focus on the morphogenesis of *Physarum*. *Physarum* is generally more dynamic than red yeast. The *Physarum* vascular system, what drives morphogenesis, and predation of the red yeast is easier to measure than colonies of millions of microscopic organisms. We hypothesize that, over time and space, the most desirable points in an environment change for *Physarum* as yeast is consumed and consumes slime mold detritus (robust colonies of yeast are the most desirable points). As we attempt to characterize the interaction between a red

yeast and *Physarum*, we are guided by several questions about their shared dynamic. How does a *Physarum* shape its plasmodia to most efficiently take advantage of the moveable feast of red yeast? It would be interesting if a morphometric summary statistic could be computed to quantify the fungitaxis of *Physarum polycephalum*. Such a statistic would probably be useful in the world of swarm robotics where many paragraphs have been recently written about the potential for swarms and decentralized multiagent systems to robustly navigate environments with complex spatiotemporal dynamics. Following this prompt we may ask: how does *Physarum* take advantage of a red yeast across different types of morphologies (one with a large growth front versus a vascularized tree) over the course of a week?

2.4 BEHAVIORAL ECOLOGY OF THE MYXOMYCETE

A recent monograph by Kataoka et al (Kataoka and Nakamori, 2020) investigates the stereotypical slime sheaths that many *Myxomycete* species deposit as they migrate. They demonstrate that different species of beetles in the genus *Collembola* may consume plasmodia, or the deposited slime sheath, exclusively (In the monograph, each of the two included beetle species consumes only one of the aforementioned). The deposited slime acts as externalized memory in the *Physarum* as well as a source of nutrients for other organisms. (Reid *et al*., 2013), (Kataoka and Nakamori, 2020) The authors note that, in the case of the plasmodia consuming insect, the slime mold is more likely to fragment - a single plasmodia splits up into smaller fragments. By observing the behavior of *Physarum* as well as other *Myxomycetes* the authors of the studies above make interesting and novel observations about *myxomycete+* morphogenesis as well as the behavioral ecology of *Physarum*. In our work we document a red yeast consuming a slime sheath. The slime sheath allows *Physarum* to navigate through its environment, communicate with other conspecific plasmodia, and serve as a nutritive source for a diverse array of organisms. Importantly the slime sheath provides a means for *Physarum* to establish an ecological relationship with other organisms.

2.5 DYNAMICS OF FUNGITAXIS AND PHOTOAVOIDANCE

We hypothesized that by exposing *Physarum* to certain regimes of light exposure we could influence its two-member ecology with a red yeast in an agar dish lined with oats. Block et al. have shown that exposing *Physarum* to blue light affects the frequency of the oscillatory contractions that shape *Physarum* morphogenesis (Alim, 2018), (Block and Wohlfarth-Bottermann, 1981). Andrew Adamatzky has shown that plasmodial oscillation frequency is affected when (blue) light is turned on and off. (Adamatzky, 2013). Continuously disrupting the oscillations of *Physarum* may broadly influence morphogenesis by dampening growth and the rate of transport of organelles and nutrients across the vascular network. These changes in growth and distribution of organelles might privilege some morphological motifs over others. Other studies such as (Briard *et al.*, 2020) expose plasmodia to white and blue light (separately over the course of two different experiments) for a few seconds. This is enough to bring about a stress response in *Physarum*. We expose *Physarum* to blue light at intervals of five minutes. It may be the case that the wavelength of light we use as well as the long (five minutes) exposure intervals are phototoxic. If this is the case, and we are poisoning *Physarum* with blue light, the light response we are trying to quantify will be confounded by this progressive photopoisoning. It should be noted that not all experiments were successful, sometimes *Physarum* simply does not grow. These outcomes are anecdotally more common in experiments with higher amounts of blue light exposure time. An avenue of inquiry that has not been addressed in the *Physarum* literature which we are well-placed to investigate, is the time and tempo of *Physarum* morphogenesis. Over long time horizons, how does *Physarum* grow and maneuver its plasmodia in a dynamic environment? *Physarum* behaviour over longer time horizons (days to weeks) is quite a young subfield, with only a few papers published recently, none of which characterize *Physarum*'s fungitactic behaviours. When *Physarum* can acquire resources how does it invest them in an environment with a mobile food source? Are certain morphological motifs more common than others. For instance, at the beginning of an experiment, one would expect to observe plasmodia organized as a growth front. We assume that these different types of morphologies (vascularized vs migratory, tree vs growth front) have different energetic requirements. By measuring

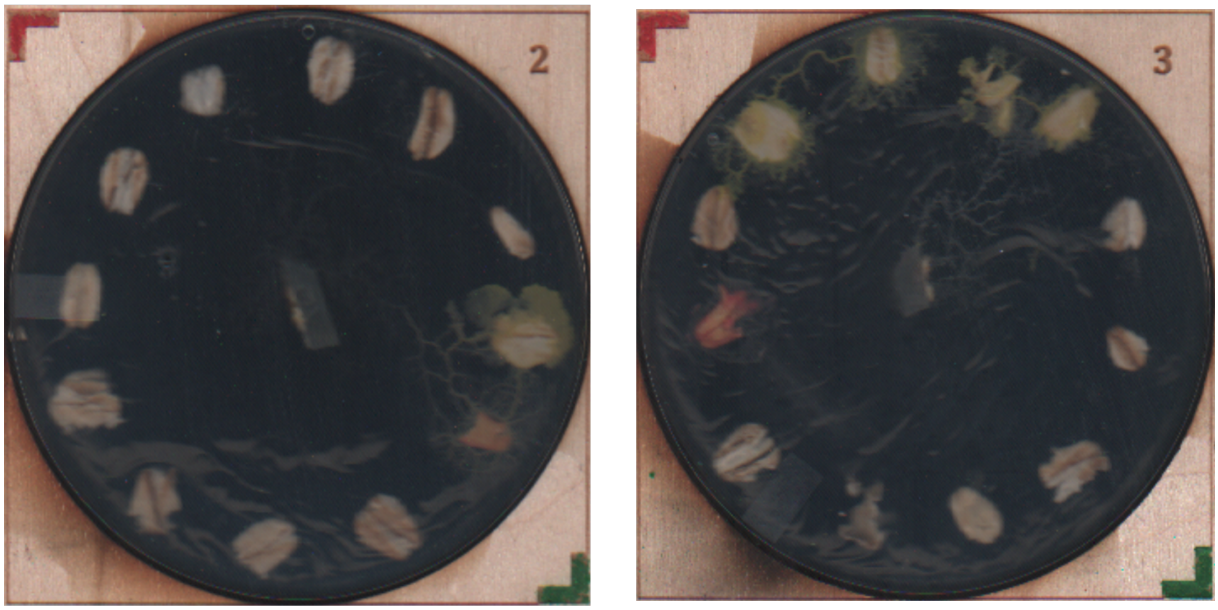

FIGURE 2.1: Different morphotypes are characterized by different actin species distribution and fluid flow organization. On the right we see a vascularized plasmodia. On the left we see a migratory plasmodia.

the mass of a growth front over time it might be possible to conclude that mass is constant and that when *Physarum* is in this migratory morphology it is not using nutrients to create more plasmodial area. *Physarum* has been shown to be efficient at partitioning its morphology to meet complex static nutritional challenges. Perhaps *Physarum* transitions between vascularized and migratory morphotypes to overcome complex spatiotemporal nutritional challenges. In Figure 2 examples of each morphotype are shown. Migratory plasmodia have a large growth front and no visible veins. Vascularized *Physarum* has ectoplasmic veins, connecting the plasmodia to different sources of food. It is less mobile than migratory plasmodia. Morphotypes provide us with a conceptual framework with which to build a morphospace. *Physarum* may habituate to a stimulus and learn to ignore it. It is also the case that, as *Physarum* navigates an environment it deposits a slime trail that may act to repel or attract *Physarum*. In the following paragraphs we describe the current knowledge on these two facets of *Physarum* behavioral ecology and how they might effect our efforts to quantify *Physarum* morphogenesis and fungitaxis in a morphospace framework. As *Physarum* adapts to and changes its environment, might it also be biasing itself to certain types of morphologies?

2.6 *physarum* HABITUATION

Physarum habituation Recently papers have described *Physarum*'s ability to repress its response to negative stimuli (such as salt and caffiene) if these stimuli are associated with sources of nutrition. (Boussard *et al.*, 2019) Papers have also shown that *Physarum* can transfer this learned response to other plasmodia when it fuses with them. (Vogel and Dussutour, 2016) The biological substrate of *Physarum* acclimation seems to be different from its ability to compute shortest paths and physical processes in morphogenesis as described by Ray and Alim.(Alim, 2018), (Ray *et al.*, 2019) In Vogel et al. plasmodia are conditioned to a particular stimulus. These conditioned plasmodia may be cut up and fused with plasmodia that have never encountered the relevant stimulus. As the conditioned and unconditioned plasmodia fuse together they form a conditioned plasmodia. (Vogel and Dussutour, 2016). This behavioural

response is driven by some biological signal contained within the plasmodial endoplasm. (Vogel and Dussutour, 2016),(Boussard *et al.*, 2019). The habituation becomes more pronounced the longer the *Physarum* is on the specific substrate. It would seem that *Physarum* habituation is driven by the absorption of some environmental element into the endoplasm. Because *Physarum* is unable to take light into its vasculature. We hypothesize that global light exposure will elicit a consistent morphological response (an hour of low light followed by an hour of high light induces a migratory phenotype in *Physarum*). The above papers posit that a modified stimulus response involves *Physarum* incorperating a stimulus such as salt into the fluid that flows through its veins. *Physarum* morphogenesis is preceded by some biochemical processes, modification of these processes perhaps by light could effect patterns of morphogenesis. Less speculative is the fact that *Physarum* can modify its environment in ways that can change its future patterns of exploration. Like habituation this behaviour could also influence *Physarum* morphogenesis and confound our attempts to measure the effects pf light exposure on *Physarum* morphogenesis.

2.7 EXTERNAL MEMORY IN *physarum*

External memory in Physarum Recently papers have demonstrated that *Physarum* may modify its environment so that it may better avoid obstacles and find regions that contain food. Dussotour has shown that *Physarum* can navigate environmental obstacles such as barriers around food, by depositing a slime sheath in areas that they visit (Reid *et al.*, 2013). When *Physarum* travels across its environment it leaves behind portions of its actin skeleton and other unidentified components. Reid et al. demonstrate that *Physarum* tends to avoid areas where they have deposited slime sheathing. By avoiding previously explored areas plasmodia are more efficiently able to explore their environment and navigate around obstacles through a process of elimination (unproductive paths are entombed by *Physarum* slime sheath). Recently in a monograph from Briard, et al. (Briard *et al.*, 2020) the topic of external memory and its relationship to slime molds deposition of its extracellular slime sheath was further investigated. The new observations in (Briard *et al.*, 2020), with Dussutour

as a senior author, essentially correct the previous paper(Reid *et al.*, 2013) also by Dussotour. In the paper, the authors demonstrate that not all slime sheaths give off an avoidant signal to other conspecific/clonal plasmodia. The signal of the slime sheath is highly correlated to the stress state of the plasmodia. Well-fed, unperturbed plasmodia leave trails that are attractive to other conspecifics. Stressed plasmodia leave repellant slime sheaths. The authors show that *Physarum*, when given a choice between regions explored by stressed plasmodia, regions explored by unexposed plasmodia, and unexplored regions, will most likely go to the region with slime sheath deposited by the less stressed plasmodia. Dr. Alim says in her paper 'Fluid flows shape organismal morphology' that physical forces may feedback into biochemical reactions to trigger complex spatio-temporal dynamics…currently more and more observed in the morphogenetic(Alim's spelling) processes.(Alim, 2018) It is possible that a similar feedback loop could be created when *Physarum* consumes the red yeast. As (Briard *et al.*, 2020) shows, a well-nourished *Physarum* deposits an attractive slime sheath. As the plasmodia migrate in the environment and consume yeast, it might tend to leave more attractive slime sheath nearby sites of yeast predation. This site of slime sheath deposition could be consumed by the red yeast as well as serve as a future attractive stimulus. If not all slime sheath is consumed by the red yeast, the sheath would attract the *Physarum* to the yeast once again. It is tempting to hypothesize that this stigmergic slime sheath could serve as a substrate for sustained successional ecological interaction. More ominously (for our attempts to characterize photomorphogenesis) the spatial distribution of the slime sheath could limit the morphological transitions *Physarum* can make by repelling or attracting *Physarum* to specific areas of the dish. As times goes on in the experiment and as more of the dish is coated with the slime sheath patterns of plasmodial migration and taxis may become increasingly correlated with the spatial arrangement of the slime sheath and canalized in a self reinforcing loop. This type of process may confound traditional morphometric analysis. Aside from the external memory the plasmodia creates with its slime sheath and the peristaltic pumping, there are a couple of other biological processes that shape *Physarum* morphogenesis. These procceses include actin synthesis and mechanotaxis. Both of these processes can be partially

observed in the morphology of *Physarum* but clearly have a biochemical component. In describing these processes we articulate additional ways biochemical processes manifest themselves across longer timescales as morphogenic processes.

2.8 *physarum* actin synthesis and taxis

Physarum actin synthesis and taxis In a 2018 paper by Oettmeier et al. "Form follows function: Ultrastructure of different morphotypes of *Physarum polycephalum*", the ultrastructure of different plasmodial morphotypes are examined. (Oettmeier *et al.*, 2018) In the context of this paper, morphotype refers to the location of blebs along the perimeter of the plasmodia. A bleb is a bulge in the membrane of a cell. In the case of *Physarum*, a bleb can be thought of as the location of the growth front / leading edge of the plasmodia. Oettmeier et al. choose to analyze three different blebbing geometries in *Physarum*. The first is a microplasmodia; this type of plasmodia is grown in liquid culture and is quite small. A microplasmodia may bleb anywhere on its surface. The plasmodia exists in an environment different from the one we are interested in, so we are not going to focus on it (liquid culture). The other two morphotypes; mesoplasmodium and macroplasmodium are roughly divided into two different quadrants of adhesion and blebbing. Each of these morphotypes moves in one direction. In the case of the mesoplasmodium, the growth front/bleb/ leading edge is focused and singular, while in the macroplasmodium the bleb is more diffuse and not contiguous along the plasmodial perimeter. When the mesoplasmodia migrate all of its mass moves as one. When macroplasmodia explores its environment the area of the plasmodia does not remain constant; expansion occurs behind the macroplasmodial growth front creating more plasmodia. Across the two morphotypes the synthesis and organization of actin are also different. Oettmeier et al. describe two types of actomyosin structures; endoplasm and ectoplasm. Ectoplasm is stationary, high in F-actin content, contractile, solid, porous, and has a high viscosity. Ectoplasm forms the walls of the veins through which the Endoplasm/cytosol flows through. (Oettmeier *et al.*, 2018) Endoplasm has high G-actin content, flows, is plastic, has no pores, and has a low viscosity. Endoplasm flows through the ectoplasmic veins of *Physarum*. Endoplasm can be

thought of as cytosol. (Oettmeier *et al.*, 2018) These two states of actin organization are not permanent and can be interchanged by sufficient pressure as well as by actin polymerization or depolymerization. The Mesoplasmodium as it moves around its environment maintains a constant size and has a well defined growth front, when it migrates the whole organism moves as one (less adhesion). (Oettmeier *et al.*, 2018) In a macroplasmodium mass is created; as the growth front moves forward a layer of solid ectoplasm is present at the leading edge and in its wake a membrane full of endoplasm is left (more adhesion). Mass is created in the wake of the growth front. These structural classifications based on observations of actin architecture provide a useful framework to describe different sorts of morphotypes that we might encounter in our fungitaxis experiments. By measuring the age, mass, and morphology of plasmodia we can link these observations of form and function to behavior. From these observations, we expect that environmental features that interact with the fluid flow of the *Physarum* might influence taxis behavior.

2.9 *physarum* MECHANOTAXIS

Physarum mechanotaxis Murugan et al. (Murugan *et al.*, 2020) demonstrate that *Physarum* preferentially migrates to regions of an elastic medium that are under more strain. The more compact and dense the regions are, the more likely the *Physarum* is to migrate to them. The authors propose that the peristalsis that drives the fluid flow interacts with a strain gradient created by the objects pushing down on the media in these regions. Like a glacial stream going down a mountain wash, *Physarum* follows the path of least resistance to the region with the greatest strain. Interestingly the authors show that by inhibiting stretch sensitive ion channels involved in mechano-sensation they were able to diminish the mass preference. For their analysis the authors use a boilerplate random forest model where they feed in images of *Physarum* and predict where the *Physarum* will end up going – to a region of high strain or low strain. To make sure that they are not cheating, when training the model, the authors crop the parts of the image that contain the masses, and also flip the images. These models have some predictive capacity after a certain time point in the experiments. The ion inhibition

data would seem to suggest that, at first, there is some sort of biochemical cascade that can translate the vibrations (generated by peristaltic contraction) influenced by the environment into a morphogenic response. This biochemical cascade theory would also explain the improvement of the random forest model with time; after a period of time the biochemical cascade mounts a morphogenic response that the random forest can detect. Without this cascade, a morphogenic response cannot be initiated and *Physarum* is less able to taxi toward regions of high mass and strain. *Physarum* morphogenesis is driven by some sort of biochemical signaling regime in addition to fluid flow and other physical mechanisms.

CHAPTER 3

Experimental design

3.1 EXPERIMENTAL PIPELINE

An experimental pipeline was designed to create a training corpus for the triplet loss and FNN-LSTM neural networks. While these networks produced pathological results we were still able to observe interactions between *Physarum* and a red yeast across experimets. This pipeline was created by and improved upon by Zeth Dubois and Leo Epstein. Zeth created the imaging apparatus and Leo designed the experiments. Each experiment contained the same components and was arranged in the same way. On a non-nutrient agar plate 12 oats were arranged on the perimeter of the plate like numbers on the face of a clock. The oat at the 6 o'clock position was innoculated with a red yeast. With this consistent spatial arrangment it was beleived that we could more easily quantify the effect that varying the probability of light exposure across time would have on morphogenesis. Oats were used as food/fuel to prolong our observations of *Physarum* and a red yeast. While some might say that oats interfere with our attempts to quantify *Physarum* yeast interactions papers have shown that even in their presence interactions between *Physarum* and micro-organisms occur and do so at a great rate.(Cohen, 1939). Oats are inert and were consistently placed across all experiments, this consistency makes it unlikely that they confounded our observations of *Physarum* fungitaxis. An imaging platform was created to acquire time lapses of *Physarum* morphogenesis at a medium throughput. With this scanner set up six plasmodia could be imaged simultaneously. Atop these scanners we mounted LEDs which could turned on or off at intervals of five minutes. The imaging platform was designed to be low cost and so it may be easily modified to overcome an shortcomings we found in the generated data. Iterations of the experimental pipeline solved various imaging and data processing challenges. *Physarum* is grown in 100mm polystyrene petri dishes on non nutrient agar atop a flatbed scanner(Canon Lide200). On top of the scanner a wooden template is placed

with six spaces for polystyrene dishes. An open source API, SANE (Scanner Access Now Easy) provides command line control of the scanners. A bash script using the cron daemon was written to schedule scans at regular intervals. This script was extended to control a blue light,turning it on and off at regular intervals. The CRON daemon enables runtime light status assignment (on or off). Instead of a regular petri dish lid, dishes are fitted with a blacked out funnel, its sloping walls prevent condensation from forming. The black finish prevents outside light from reaching the *Physarum*. A fitting ontop of the black funnel allows us to mount the DIY RGB LED and expose *Physarum* to blue light. On top of these cones another template is placed so they do not fall over or shift over the course of the experiment. Cronjobs, light management, and file management are run using a raspberry pi. BASH scripts, run on the pi, coordinate the aforementioned. Atop of everything another cardboard enclosure is placed to ensure further stability as well as protection from any other light source that might disrupt the experiments. The experimental setup is shown in figure five. With our experimental set up we are able to observe *Physarum* morphogenesis over periods of time substantially longer than reported by others in the field.(Westendorf *et al.*, 2018),(Reid *et al.*, 2012) The actinic spectrum that scanners use is of a very short wavelength and may have damaged the biomolecules contained within the *Physarum* endoplasm. A plastic wavelength filter could have been placed atop the scanner to filter our particularly short wavelengths.

3.2 EXPERIMENTAL OVERVIEW

In our study, we observe *Physarum* morphogenesis across the scale of days. The images we acquire to analyze *Physarum* morphogenesis are taken using a flatbed scanner. Scanner images are segmented by hand using the tool ILASTIK.(Berg *et al.*, 2019) These hand segmented images are combined to create a curated ground truth training set for a U-Net. Included in the training corpus are plates with a large quantity of healthy plasmodia, plates with a large quantity of dead plasmodia, plates with a large quantity of yeast, and plates in which these three classes interface. Plates close together in time are also included. By including these close-in-time plate pairs

FIGURE 3.1: A photo of the imaging apparatus.

there will be more frame-to-frame class consistency. Despite these strategies, the trained U-Net model is not perfect. The training dynamics of U-Net are not so well understood. Measuring and understanding the dynamics of robustness in neural networks is a very active area of research that requires some mechanistic knowledge of the neural network learning process, which the field does not have. No optimal U-Net training regime exists. We suppress U-Net frame-frame variability by taking two adjacent frames and comparing them. Any pixel with the identity of *Physarum* in both images is labeled *Physarum* in the denoised new image. Pixels pairs with only a single *Physarum* identity are discarded/ changed to background. This process results in fewer noisy pixels along class boundaries and fewer scanner artifacts. It is now easier to identify short term trends. This filtering is carried across all time series. We are also interested in measuring morphogenesis over longer temporal horizons (more than a couple of frames). In this context other error suppresion rules are used. Across fifty consecutive frames pixel values are summed along the time axis and the median plasmodial age, variance, and mass are computed. Several frames at the beginning and the end of the fifty frame window are used to make binary masks based on presence or absence of pixels with the idenity of active plasmodia. These masks are compared with a presence absence mask of *Physarum* across the rest of the temporal window. These comparisions give us some metric of *Physarum* growth and retraction.

3.3 MEASURING THE MACROPLASMODIAL AND MESOPLASMODIAL

How might *Physarum* adapt its morphology as it forages for a spatiotemporally variable resource. Most if not all recent papers on *Physarum* morphogenesis have been focused on analyzing *Physarum* growth and exploration in a static environment. I believe that this static environment might provide a somewhat misleading picture of the behavioral ecology of *Physarum*. An example of this is *Physarum* fragmentation which has already been mentioned. Tiny fragments of plasmodia may be produced by predation and eventually aggregate to form a mesoplasmodia. The dynamics of predation have a marked impact on *Physarum* morphogenesis. In an environment with

a spatiotemporally variable but rich food source (a red yeast) a *Physarum* may more efficiently forage in a mesoplasmodial form with a huge growth front then by using a small pseudopod as macroplasmodia. Static versus dynamic environments may favor different morphogenic regimes such as fragmentation or migratory behaviors. Our experimental design increases our odds of observing novel forms of *Physarum* morphogenesis and may also allow us to reinterpret older observations of *Physarum* biology. We are especialy interested in quantifying and testing hypotheses dealing with the behavioral ecology of *Physarum polycephalum* With our experimental pipeline we attempt to create a datdaset that can be used to characterize *Physarum* and red yeast interactions. We hypothesize that they interact in a way that can be captured across the image time series we produce. We performed dozens of experiments hoping that we would be able to test our hypotheses about blue lights effects on *Physarum* taxis, and its theorized downstream effects on *Physarum* yeast interactions. We expect that, in some cases, there should be a sort of predator-prey interaction akin to a Lotka-Volterra model between the *Physarum* and a red yeast .Titrations of light frequency per hour might effect the dynamics of this predator prey interaction. A predator-prey interaction is feasable between these two organisms, as *Physarum* consumes the red yeast, red yeast consumes the slime sheath that *Physarum* leaves behind, and finally the *Physarum* migrates around the dish, leaving in its wake the slime sheath which can be consumed by the red yeast. While this sort of interaction could happen it probably won't be observed across experiments with both yeast and *Physarum* because unmixed systems have very chaotic dynamics. *Physarum* will not always be able to find yeast to eat, and yeast will not be able to grow on inert slime sheath. When the needs of the organisms are met interaction takes place are met we may measure the dynamic via wavelet analysis. This is a technique commonly used by researchers interested in analyzing an oscillating system such as Lotka-Volterra. For instance, researchers studying plankton-rotifer interactions use wavelet analysis to analyze the dynamics of this predator-prey pair.(Blasius *et al.*, 2020) Wavelet analysis provides summary statistics that can be used to compare *Physarum* and yeast interactions across differing light conditions. In this masters thesis we gambled on having enough *Physarum*-yeast interaction data that we could measure the

interactions between *Physarum* and yeast to a degree that allows us to characterize the light response in this system (by performing stastical analysis of the wavelet analysis summary statistics). To characterize the complex morphogenic program of *Physarum* across many experiments with heterogenous imaging conditions, and the effects that fungitaxis as well as blue light exposure might have, it was neccesary to develop a robust computational pipeline.

CHAPTER 4

Computational Pipeline

4.1 COMPUTATIONAL PIPELINE

A computational pipeline was developed to measure and analyze how photo avoidance and fungitaxis influence *Physarum* morphogenesis. Series of raw images containing several distinct plasmodia were semantically segmented using Ilastik and U-Net. Ilastik interactive segmentation was used to create a training dataset for a U-Net which would in turn segment all the data.(Berg *et al.*, 2019),(Ronneberger *et al.*, 2015) Problems with the U-Net segmentations could be fixed with additional or modified Ilastik segmentations. Once all raw time series were semantically segmented with U-Net they were split apart into individual segmented time series that contained only a single plasmodia. These separate segmented time series were then analyzed with varying levels of success using neural networks such as FNN-LSTM(Gilpin, 2020), Triplet loss(Hermans *et al.*, 2017), and more traditional methods such as morphometric feature engineering and wavelet analysis(Blasius *et al.*, 2020). With this diverse set of methods, we tried to first quantify *Physarum* morphogenesis, and then use the measurements to better understand how photoavoidance shapes *Physarum* morphogenesis. How do different regimes of light exposure change the dynamics of *Physarum* morphogenesis and how might changes in taxis effect *Physarum* predation of a red yeast? f

4.2 ILASTIK

Ilastik pixel segmentation was used to create and curate interactively labeled ground truth masks. Ilastik is an interactive random forest classifier used for image analysis. The workflow for Ilastik pixel segmentation is straightforward and fast. Portions of an image are hand segmented by the user. These segmentation annotations are then used by the Ilastik classifier to create filters with which it may classify the rest of the image.

The user may add or remove segmentation annotations from the image used to train the Ilastik classifier to improve its performance. Iterative annotation is carried out until a satisfactory segmentation is created by Ilastik. A segmentation is considered sufficient when a large portion of the active plasmodia are accurately segmented and its vascular morphology well preserved. Holes in the plasmodial vasculature are well defined, and blebbing across the perimeter looks plausible. To create the filters required to train the Ilastik classifier, portions of our images were labelled as one of the following classes: protoplasm rich *Physarum*, protoplasm poor *Physarum*, red yeast, oats, and background. In Figure 3 examples of these classes and their annotations are shown. The features used in conjunction with the user annotations to create the segmentation were color intensity with a sigma of 0.3, edge with a sigma of 0.7, and texture with a sigma of 0.7. Ilastik uses these features to "look for patterns" in the curated annotations. Sigma refers to the width of the Gaussian kernel used to smooth the image before application of the feature filters. The three sigmas chosen for each filter were the smallest available. With a smaller gaussian kernel it is easier to produce a semantic segmentation that faithfully captures the delicate structure of the *Physarum* vasculature and more faithfully captures finer detail in the semantic segmentation. The segmentation annotations used to train the ilastik classifier were constructed by finding circular regions (with a radius of 31 to 61 pixels) of the image that contained as many of the aforementioned classes, as possible and fully annotating them. By fully annotating these circles we can define the boundaries between each of the classes which may appear very similar. One such boundary would be between the red yeast and depleted protoplasm. Both of these classes have the same translucent white color, differing only in shape and texture. This approach to annotation advances our stated goal of capturing the nuances of the *Physarum* vasculature by accurately capturing the shape of objects from each class and the shape of the interface between classes as well as the texture of the different features. Ilastik provides an easy and rich user interface to carry out interactive segmentation with a powerful random forest backend.

4.3 DECISION TREES AND RANDOM FORESTS

Until recently Ilastik has exclusively used a random forest backend; as its maintainers say this family of algorithms are robust and can be trained with relatively sparse annotations. As convolutional neural nets have become increasingly popular, the maintainers have been building out backend infrastructure to allow the substitution of the random forest classifier with a U-Net. A random forest belongs to a family of algorithms called decision trees. A decision tree, is at its most basic, a collection of binary tests organized in a tree structure. Each interior node of the tree is labelled with a test. (Geurts *et al.*, 2009) This test takes a value from the input and compares it to a threshold. Each terminal node of the tree is labeled with a class. To classify a new object it is propagated into the tree from the top node. When a terminal node is reached, the class label of the terminal node is assigned to the object. In the context of Ilastik, an object is a pixel. In a decision tree, the criteria for splitting nodes, generally stated, is to find the test that creates leaves with the least variance in the resulting nodes. By creating enough node-tests it is possible to classify all training data correctly. When creating these trees it is essential to avoid overfitting.To lessen the problem of overfitting, rules can be created to stop node splitting / tree growing. Rules can also be created to reduce the number of nodes in existing decision trees. To moderate tree growth during training, a halting rule can be used that measures the increase in accuracy across cross-validation data sets as new nodes are added. When a point of diminishing returns is reached with regards to node creation and increasing CV accuracy; tree growth is stopped. The inverse of this growth halting rule is used during pruning after a decision tree has been constructed. When a tree is pruned we ask which nodes-rules may be removed with the lowest impact on accuracy across cross-validation sets. Error pruning is the most classic way of regularizing trained decision trees. Tuning the pruning and growth stopping hyperparameters is a non-trivial task. The distribution of classes and their associated features across different data sets make it challenging to know when you have reached a plateau of accuracy. For instance, class imbalances might have a large effect on the hyperparameters governing tree pruning and tree growth halting. Modifications to classical decision tree algorithm have been made to avoid the thorny issue of

hyperparameter selection. With a very large decision tree it is possible to classify all data from the training set very well but have performance suffer greatly on unseen data. This is called overfitting. Overfitting occurs when a model has learned spurious features to classify the data. Related to the concept of overfitting is information gain. Information gain, in the context of decision trees, refers to the way a rule affects the class distribution in a dataset. The information gain of a rule can roughly be defined as the amount it reduces class variance in the resulting leaf-nodes further along in the tree. Information gain is not always an important means of measurement. If each data point has a unique feature for a particular class then the information might be very good, but the corresponding test could not be usefully applied to unseen data. Ross Quinlan proposed that by taking into account the size of the branches created by an upstream/up-tree test, you could qualify the information gain by some measure of future use.(Quinlan, 1986). A decision tree with small branches will probably be prone to overfitting whereas a tree with large branches and tests that produce many data points will be more robust. Several algorithms solve the problem of hyper parameter selection in decision trees. In random forests, bootstrapping and random tree growth make the decision tree approach much more robust. By aggregating many decision trees at once it is possible to substantially boost classification accuracy without substantially increasing overfitting. Ilastik creates an ensemble or forest of trees via the random forest algorithm. Each tree grown in the random forest is built from a bootstrap sample. Each node of the tree is chosen by picking N attributes randomly amongst all input attributes; an optimal test is found for each of these attributes. The best rule generated from these tests is used to split the node. Repeating this process across trees and nodes allows you to efficiently sample the feature space and construct a robust decision surface. Boot strapping the training sample allows you to combat the problem of class imbalance; producing datasets with differing class compostions. Decision trees were very popular in the early 2000's; lately, they have been eclipsed in popularity by neural nets. Despite this, they are still being improved upon and are an active area of research. Randomer forests developed by Joshua Vogelstein's group are a recent addition to this family of algorithms. Randomer forests use random collections of linear combinations of features to improve accuracy and

robustness. They achieve state of the art performance across one hundred different data sets. (Tomita *et al.*, 2019).

4.4 U-NET

While the ilastik classifier is quite robust across an image, there are several weaknesses inherent in the method that make it difficult to use at the scale we require. These issues include runtime of the classifier; when you add more training data-class/pixel examples/nodes to the classifier, runtime(even during training!) can take minutes to tens of minutes. Training a robust model is inhibited by memory and runtime constraints. It is also the case that accuracy drops when images are classified by random forests trained on different time series and scanners. This change in performance may be associated with artifacts that are unique to the time series (plasmodial thickness, vascular architecture, as well as the spatial organization of *Physarum* and yeast interaction) or the scanner associated with the time series. To overcome these issues we interactively train several Ilastik classifiers whose outputs we can collate in a training corpus for a neural network with a U-Net architecture. This neural network architecture has become very popular in the field of biological image analysis recently due to its ease of use and good performance across many different semantic segmentation tasks. By taking segmentations from many different Ilastik classifiers we hope to create a more robust pixel classifier. Simply put we are able to quickly train several Ilastik classifiers to accurately segment a single image. These images can have widely differing abundances of semantic segmentation labels as well as different brightnesses, luminosities and unique scanner artifacts. With a U-Net we may distill these many random forests into a single more robust model. This approach of training a neural network on somewhat noisy data may be considered as a form of augmentation a powerful technique that increases the performance of neural networks in terms of accuracy and robustness. In the following we describe the ways in which the U-Net architecture and data augmentation interact to produce useful semantic segmentations that can be used to measure complex biology from image data. A U-Net is a fully convolutional neural network with an autoencoder architecture where

pooling and upsampling layers of equivalent resolution are connected by skip connections.(Ronneberger *et al.*, 2015) Pooling is a process in which the feature map (in this case 2D array) is reduced along both axes by some factor. For example 10x10 => 5x5, if the stride of the pooling is 2. In the aforementioned case a 2x2 convolutional window will be applied across the 10 by 10 feature map and the average, maximum, whatever other type of operation you want, can be selected 5x5 times. (Kauderer-Abrams, 2017) Upsampling carries out the above operation in the inverse; interpolating a 5x5 2d feature map to a 10x10 feature map. Some evidence exists that pooling and convolutional filter size help with translational invariance/ robustness but were secondary to the importance that data augmentation played(Kauderer-Abrams, 2017). Skip connections may allow the network to propagate information across the pooling and upsampling bottleneck, allowing more layers in the neural network to learn. Drozdal et al observe that by adding skip connections to a fully convolutional resnet they are able to get a model to converge faster and have more layers in the model be updated with each epoch.(Drozdzal *et al.*, 2016) Many U-Net models, including the one used by us implement extensive data augmentation. Augmentation strategies include rotating images, shuffling channels in images, and rotating channels in images. Wang et al. have shown that data augmentation (rotating the training images) can greatly increase the performance of a U-Net. In the original U-Net paper the authors mention that strong augmentation allows the U-Net to learn invariance to the deformations introduced in the augmentations. (Wang *et al.*, 2019) While important features of the U-Net architecture have been shown to increase neural net performance, when used together and separately, no papers have been published detailing how a U-Net works and WHY its associated architectural features and training strategies are SO effective. More broadly the impact of neural-net architecture and training regime on neural network performance are not well understood. Despite this uncertainty about the inner workings of the U-Net, we are able to successfully train one on a small number (10 out of 10,000) of raw images and their Ilastik interactively annotated ground truth counterparts. With a trained U-net we are able to generate semantic segmentations of protoplasm-rich *Physarum*, protoplasm-poor *Physarum*, the red yeast, oats, and the background across all experiments.

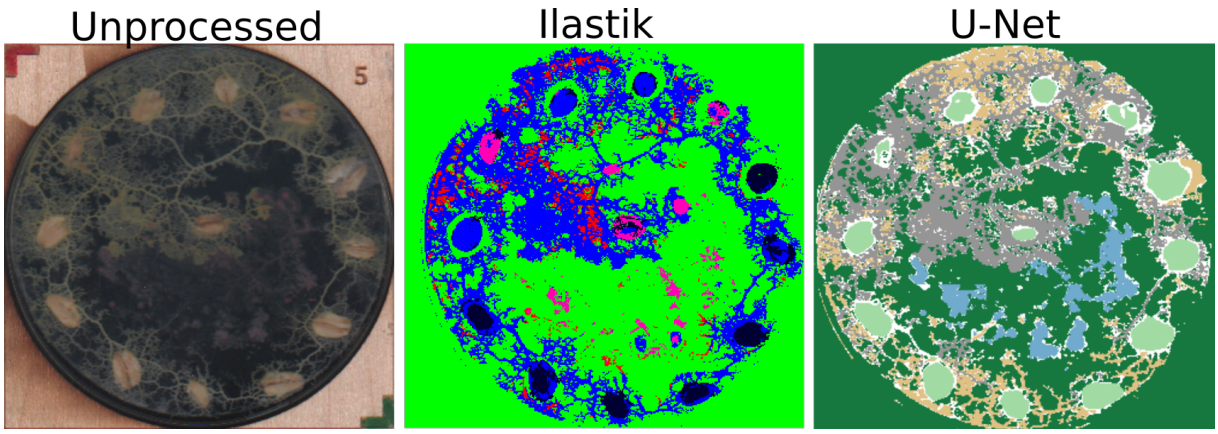

FIGURE 4.1: In the side by side comparison of the two segmentation strategies and the ground truth images we can clearly see that U-Net more faithfully captures the vascular architecture of *Physarum*; active and inactive, than its Ilastik counterpart. While neither segmentation algorithm can identify all of the yeast colonies, U-Net captures many more than Ilastik.

4.5 DATA SORTING

With these pixel-by-pixel semantic segmentations we set out to measure biological processes associated with *Physarum* morphogenesis. Each of the six plates across each scanner run were seperated into a segmented time series with each plate annotated with the classes of plasmodia poor, plasmodia rich .... etc. To detect the circular plates we used our knowledge of the experimental set up. In each scanner image there is a light wooden frame which has holes for six polystyrene plates full of media. These polystyrene agar dishes are under a black cone that shades *Physarum* from outside illumination and prevents too much condensation from forming as *Physarum* perspires (not much is known about *Physarum* respiration). These shade cones create six black circles against a light wooden background. These six circles have a similar radius and color. By using a hough circle detector with the radius, number and distance between the centers of these dark circles as hyper parameters we find the coordinates of each plate early on in the experiment and use these coordinates to divide an image of six dishes into six separate images of one dish. Assuming that these plates do not move around too much we may use the coordinates from the hough circle detector to create six separate plasmodial time series for each six plate scanner run. As each experiment has a slightly different placement of the wooden frame the hough circle detector is run one time for each experimental run. A substantial amount of work was put into creating an imaging platform with which we could keep *Physarum* healthy for a substantial period of time, while also observing its morphogenic behavior.

4.6 DATA ANALYSIS AND RESULTS

With the time series of single plasmodia, more involved computational techniques were implemented to analyze the morphogenesis of *Physarum* as well as its fungitaxis and photo avoidance behaviors. Some of these techniques met with more success than others. A brief overview of the techniques used to analyze *Physarum* morphogenesis includes metric learning, implementing an FNN LSTM, wavelet analysis, and morphospace analysis. While not always successful or productive in the way

that we wanted; these exercises provided valuable insight into the limitations of the segmentation pipeline as well as the limitations inherent in the dataset (limitations of experimental design). We observe for the first time *Physarum* fungitaxis behavior and are able to propose new avenues of inquiry and contextualize old observations in a new light. We were not able to find any correlation between light exposure regime and morphogenesis. We observe particularly strong batch effects that may have hampered our search for such a correlation. Nutritional status as well plasmodia origin may have confounded our effort to correlate light exposure regime to *Physarum* morphogenesis.

4.7 FNN-LSTM

An analysis of *Physarum* fungitaxis and yeast predation was undertaken using William Gilpin's false nearest neighbour LSTM network.(Gilpin, 2020) We believed that we could characterize the dynamics of *Physarum* and red yeast interaction by analyzing changes in *Physarum* mass over time. We thought we might be observing an oscillating ecological interaction (a predator-prey Lotka Voleresque type dynamic). These sorts of interactions are classical examples of strange attractors. In the paper by Gilpin "Deep reconstruction of strange attractors from time series" a recurrent autoencoder with a regularizer that uses the false nearest neighbours statistic is described. Using this network you may reconstruct the latent space of a strange attractor from a univariate time series without having to test for the appropriate time lag and the most informative dimensionality of the latent space; these tasks are obviated by the regularizer. To calculate the false nearest neighbours of a point you measure the distance of the point to its neighbours in an n-dimensional space and then do the same in an (n + 1) dimensional space. By calculating the ratios of the distances of the point and its neighbours in the n n + 1 dimensional spaces you may find which pairs are false nearest neighbours. Pairs with larger ratios are probably false nearest neighbours and ones with smaller ratioes are probably real nearest neighbours. Using this statistic as a regularizer, the neural network is able to create a more informative embedding of a dynamical system by deemphasizing uninformative latent variables (as measured by nearest neighbours attributed to that dimension). Using the FNN

LSTM network it is possible to reconstruct and visualize the dynamics of the strange attractor/ dynamical system from the time series Hankel matrix. Despite the neat idea behind the neural network and its clever implementation we did not manage to produce any informative embeddings using this method on univariate time series of cumulative *Physarum* area and have a couple of ideas as to why. In Figure Six we see that the FNN-LSTM was unable to reconstruct any strange attractors in the latent space. This may be because there is no oscillating system present in the time series, the data is nonstationary, or there are simply not enough oscillations present in the time series for an informative latent space to be reconstructed. We have observed that *Physarum* and yeast may in fact share an oscillatory dynamic, the fact that we could not reconstruct the strange attractor driving this dynamic suggests that *Physarum* and yeast interactions take place at a specific spatial scale. The univariate time series fed into the FNN LSTM may contain several morphogenic behaviours of *Physarum* that when observed together in a univariate time series cannot be distinguished.

4.8 SUCCESIONAL DYNAMICS OF *physarum* AND A RED YEAST

Succesional Dynamics of Physarum and a red yeast The spatiotemporal dynamics of *Physarum* and a red yeast's interactions are complex and difficult to capture. At the start of each trial *Physarum* and Yeast are placed in different areas of the dish. This means that there is usually significant time lag before the two organisms interact. When *Physarum* interacts with yeast, we have observed that it does, in fact, consume it. *Physarum* may invest the energy it has harvested from yeast, as well as oats into many morphogenic programs. The two morphogenic programs which that are most easily measure are growth and migration. Fitting these complex processes into a single time series is a challenging task. Migration and growth might obscure *Physarum* and yeast interactions in a univariate time series of *Physarum* mass. *Physarum* and yeast interactions may take place in a very small portion of the dish. While we have gathered data of *Physarum* sweeping over expansive lawns of a red yeast this is probably not the most common form of interaction between the two species. Red yeast takes a long time to grow and *Physarum* motility is influenced by age and

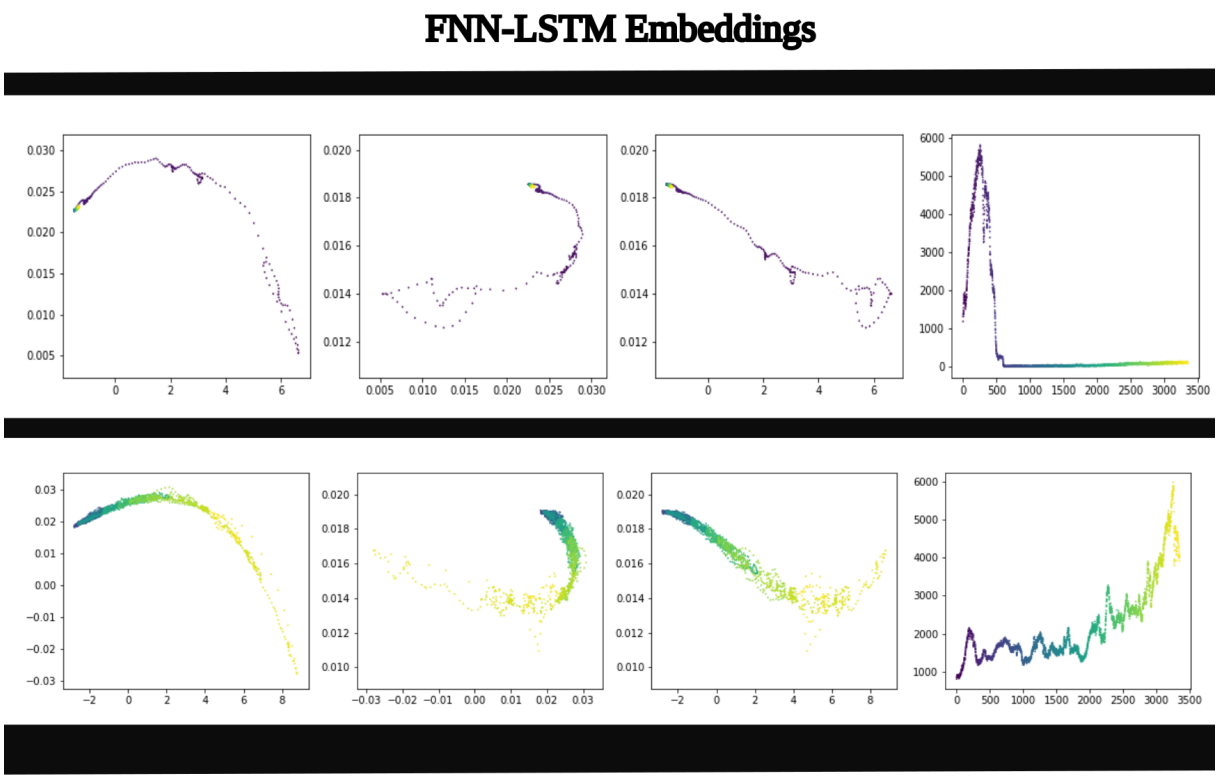

FIGURE 4.2: Non stationary dynamics of *Physarum* growth make it difficult to analyze using the FNN-LSTM architecture. First three plots are projections of the latent space. The fourth plot is a plot of *Physarum* frequency across time. Across all plots, time is encoded with the viridis palette. As time goes on the color becomes warmer. There are approximately 3500 five minute intervals. each point is the mass of a single plasmodia across time

nutritional status. A more robust way to measure the oscillatory nature of *Physarum* yeast interactions would be to measure the regions in a dish where *Physarum* and yeast both occur across the whole time series. By doing so you are more likely to be observing *Physarum* yeast interactions especially if there are sustained oscillations between the two classes. Future work would take the spatial heterogeneity of these interactions into account. In training our FNN LSTM, we are reminded that when measuring a spatiotemporal process it is important to take into account both the spatial and temporal dynamics. A succesional relationship could be obscured by other biological processes in a univariate time series that measures *Physarum* mass over time (no literature exists that says that *Physarum* can not migrate and consume yeast at the same time). While no informative dimension reduction was obtained, the process of creating and cleaning the plasmodial area time series with which the FNN-LSTM model was trained, led to novel observations about *Physarum*-red yeast interspecies dynamics. Across specific spatial scales, *Physarum* and the red yeast interactions have an oscillating dynamic. By measuring areas where both *Physarum* and Yeast occur across the time series, we can focus our measurements on the oscillating dynamic. If we include areas outside this shifting cline of interaction, we may capture in our measurements *Physarum* taxis or its behavior of approximating shortest spanning trees between food sources. Neither of these processes are stationary or cyclical as demonstrated by our failure to train an FNN-LSTM model that relied on these assumptions. The real Irish GDP is concealed by corporate tax evasion, so too are red yeast - *Physarum* dynamics concealed by *Physarum*s deep behavioral repertoire and the spatial diffuseness of yeast colonies. By taking the interaction zone approach, we can be more confident that we are only observing yeast colonies that interact with *Physarum* and *Physarum's* fungitaxis behavior. We may better measure the time and tempo of multi-species interactions. With this approach we observe across multiple time series, from different experiments what would appear to be cyclical succession between pixels of *Physarum* and pixels of a red yeast in the zone of interaction. In Figure 7 we see that in clines of interaction, *Physarum* and a red yeast may have an oscillatory relationship. Despite this success, further analysis (pertaining to the effects of photoexposure on fungitaxis) could not be carried out due to the fact

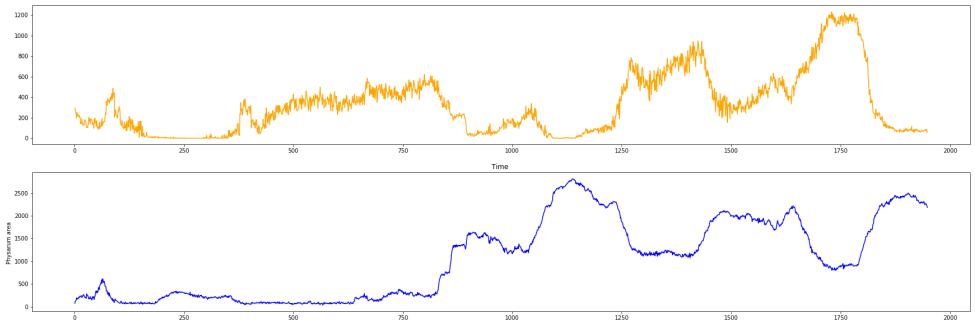

FIGURE 4.3: Top in gold: Frequency of Yeast in the successional cline over time. Bottom in blue: frequency of *Physarum* within the succesional cline. An oscillatory out of phase relationship is observed.

that *Physarum* and yeast interaction time series were not observed in enought time series. *Physarum* has shown to be fungivorous (Chapman and Coote, 1983) and others have engineered symbiotic relationships between *Physarum* and other protists (Lazo, 1961), (GASTRICH and Anderson, 2002). Having observed sustained successional cycles between *Physarum* and yeast we can speculate on the substrate that drives them. A recent paper by (Kataoka and Nakamori, 2020) mentions that a beetle may eat the slime sheath produced by the migrating *Physarum*. The slime sheath is a source of nutrition for the beetle. The clinal interactions between *Physarum* and a red yeast may be sustained by nutritive qualities of *Physarum* slime sheath. As *Physarum* leaves an area it deposits slime sheath. It is observed that the slime sheath is subsequently consumed by a growing colony of red yeast. This red yeast is then consumed by *Physarum*. While the appropriate experiments have not been carried out to determine the exact nutritive properties of *Physarum* slime sheath. The slime sheath provides a plausible explanation as to what drives this interaction. *Physarum's* slime sheath has also been shown to help clonal *Physarum* plasmodia navigate their environment. Satisfied plasmodia leave behind an attractive stimuli in their slime sheath and stressed Plasmodia leave behind a repellant stimuli in their slime sheath. (Briard *et al.*, 2020) We speculate that this slime sheath may have been consumed by microorganisms as well as beetles. Our observation could add a new layer of complexity to the biology of *Physarum* slime sheath. Following this observation we

speculate that as *Physarum* navigates its environment it occupies specific regions of a morphospace so that it may build a spatiotemporally optimal slime sheath to farm the most microorganisms. Significantly we observe, for the first time, successional dynamics between *Physarum* and another micro-organism.

4.9 TRIPLET LOSS

The next neural net approach used to analyze *Physarum* growth over time was a triplet loss embedding. It was hoped that photomorphogenesis would encode certain morphological motifs into plasmodia across different light conditions; by performing a dimension reduction technique like triplet loss we would be able to get a better understanding of how light exposure was correlated with *Physarum* growth and migration. Would different regimes of light exposure cluster in the triplet embedding space? Training goes as follows: In each batch, P classes, and K images of each class are randomly chosen without replacement. In each update three samples are run through the network. (Hermans *et al*., 2017) These three samples are the anchor, positive, and negative. The anchor is any data point you want to run through the network, the positive is a data of the same class as the anchor, and the negative is a data of a different class from the anchor. For each anchor, Hermans et al. choose the hardest negative and positive data points within the batch. The hardest negative is the point closest to anchor in the latent space that is of a different class than the anchor, and the hardest positive is the point furthest away from the anchor in the latent space of the same class. The triplet loss seeks to minimize the distance between the anchor and the positive point and maximize the distance between the anchor and the negative point. Over many updates, points are pushed around the latent space such that points of the same class cluster together and points of different classes are separate. By choosing the hardest negative and positive Hermans et al believe that an embedding will be learned at a greater rate, and more stably as only the most informative points are used. A triplet loss embedding clusters the input dataset according to a specific variable such as light exposure or *Physarum* velocity per frame. Using the triplet loss you may distill complex multidimensional relationships to lower

dimensional ones. Triplet loss is a tool for classification problems where your class examples are very sparse. Instead of learning features of each class specifically you create an embedding in which all of the classes are placed together. Identifying new data points involves feeding the point into the embedding and seeing with which points it clusters. The class of these nearest neighbours is assigned to the unknown point. An example of a problem where triplet loss may be used is be person reidentification. Instead of creating a class for every single person you simply create an embedding of all the people in your dataset. When you get new data you run it through your network and you are given coordinates in the embedding space. If you run a picture of Jose Conseco through your network he would be located close to all the other pictures of Jose Conseco. As one of the premier power hitters in major league baseball, you might expect Jose, or at least older pictures of Jose to cluster with other baseball players. As the U-net learns to split an image up into semantically labelled regions the the triplet loss can be used to embed elements into clusters of semantically labelled classes. These embeddings are very useful for characterizing relationships between your classes. The process of training triplet loss networks and more generally metric learning loss functions is difficult. The training space may collapse or the embedding may be effected by noise. If you do not have enough informative triplets the embedding will be poor. In the following I discuss the process of training a triplet embedding and touch on why ours failed. Ultimately our attempt to train the triplet loss was unsuccessful. Despite this it failed in an interesting way. Images of bioactive plasmodia were fed into the network and used to predict the between frame difference in plasmodial pixels between the image and the image that came before it in the same plasmodial time series. The embedding produced by the triplet loss when visualized with UMAP contained temporally sorted sequences of each time series. The triplet loss found that clustering the points by time and individual plate was easier than clustering by frame-frame difference is plasmodial mass. Looking at the embeddings in Figure 8, it seems that the Triplet loss is using the similarity between subsequent points across time series to cluster the data instead of some measure of plasmodial frame-frame difference. While it is possible masks of bioactive plasmodia are not enought to learn a triplet loss embedding of

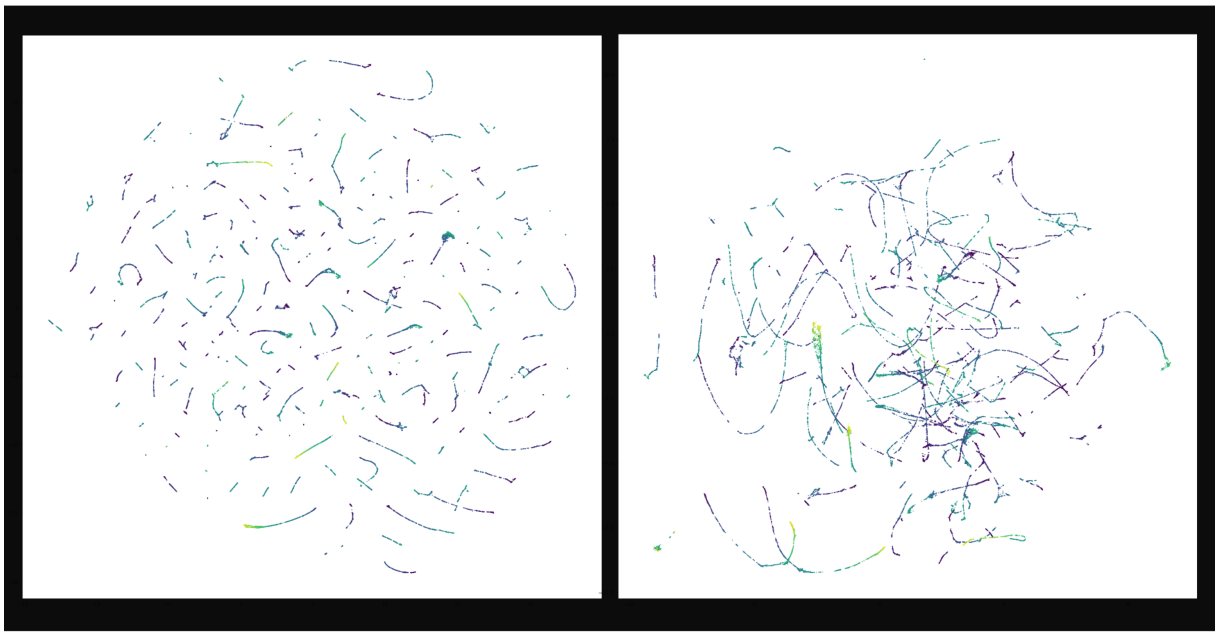

FIGURE 4.4: In both embeddings, data points separate by individual plasmodia and are temporally sorted. As with the other embeddings, time is encoded using the viridis color palette. As time goes on, the point color gets warmer. Please zoom in for easier viewing.

plasmodial frame-frame difference it is also the case that data augmentation strategies can be used to emphasize plasmodial morphology instead of the correlation between images in a time series. (Hermans *et al.*, 2017) Hermans et al. and (Dosovitskiy *et al.*, 2014) Dosovitskiy et al. have shown that data augmentation allows convolutional neural networks to learn more robust features. Several augmentation strategies that might be used are image rotation, image warping, as well as pixel jitter. Another augmentation strategy would be to strongly dilate, the pixels in the bioactive plasmodia mask so that most if not all of the "real" non-noise bioactive plasmodia pixels would be contained within one region. This region could be used to create a smaller rectangular crop that could be applied to the undilated image. Using this cropping strategy, in conjunction with the other augmentation methods might allow the triplet loss to learn a more robust embedding of plasmodial velocity by removing long range temporal correlations contained across a single plate. Ultimately these two attempts at implementing neural networks have provided us with insight about the appropriate spatiotemporal scales at which *Physarum* morphogenesis can be observed. *Physarum* plasmodial growth should probably be measured across multiple frames to overcome technical error. *Physarum* and Yeast, interactions occur at a local scale and their measurement should reflect that. Our interaction zone observations also suggest that *Physarum* morphogenesis and its behavioural modules can be localized. Vascularized plasmodia can be largely stationary while putting out many smaller growth fronts which would look very similar if we compared it to a much smaller migratory plasmodia using the measure of plasmodial velocity in terms of measuring univariate time series of plasmodial mass. By looking at the age of a plasmodia across time we can get a much better sense of *Physarum* morphogenesis and be able to differentiate between the two aforementioned morphotypes. By extracting summary statistics of *Physarum* plasmodial age over time we can get a sense of the forces that shape *Physarum* morphogenesis. We hypothesize that light and fungitaxis over some time frame, effect *Physarum* growth and migration and have designed our experiments in hopes of measuring their effects. It is also the case that *Physarum* morphogenesis can be shaped by the internal state of the plasmodial for instance plasmodial nutrition,

stress, and perhaps even the humidity in the growth chamber can have profound effects on growth.

4.10 CONSTRUCTING A PHOTO-MORPHOSPACE

The presence of photomorphogensis is tested for by correlating the change in plasmodial morphological state, with its exposure over time to blue light. If the dynamics of plasmodial morphogenesis correlate with changes in the light regime, then photomorphogenesis may have taken place. Ultimately we were unable to find a correlation between morphogenesis and light regime. Despite this I think some of the metrics used to construct the morphospace may be useful in future data analysis. I also have some hypotheses as to why we were unable to correlate plasmodial morphogenesis to the light regime. The features used to quantify *Physarum* morphogenesis include metrics such as plasmodia area, perimeter, plasmodial pixel median age, the variance of per pixel plasmodia age, the plasmodial density across convolutional windows of different sizes, the variance of plasmodial pixel age using a max projection across a temporal interval, the variance in age across the same temporal interval and the mean value of plasmodia pixels after a distance transform. While the pixel is an arbitrary unit of measurement in the context of plasmodial biology - the model and resolution of our scanners are consistent across all referenced experiments. By measuring the median age and variance of per frame of plasmodial pixels we may come to a better understanding of the cohesiveness of plasmodial morphogenesis. How many growth fronts are present across the plasmodia? Are some portions of the plasmodia more vascularized than others? While some Plasmodia migrate by moving their whole mass across the environment, others send out growth fronts from a comparatively static vasculature. Plasmodial per pixel variance in age may help us differentiate between these two modes of movement. An increase in variance and mass would indicate that parts of the plasmodia are old and others are new. A migratory plasmodia will have a more constant pixel-wise age variance. As new plasmodia is created in the growth front, older plasmodia decays. The plasmodia flux inherent in this migratory mode of exploration dampens the variance in per pixel plasmodial age and maintains

a more constant mean per-pixel age. If light were to affect the time and tempo of *Physarum* morphogenesis and exploration we may be able to detect it using these two summary statistics. Ultimately we were not able to correlate light exposure regimes with our measurements of morphogenesis. While there were promising aggregate trends. For instance, as the frequency of light exposure over some time period rises, the plasmodial density rises then falls. This trend in plasmodial density was not present when individual plasmodia were measured. As light exposure changes across time so does plasmodial nutrition. While the dynamism of *Physarum* morphogenesis makes it hard to correlate it with a time-varying stimulus, the fact that it can habituate to stimuli adds another layer of complexity. (Boussard *et al.*, 2019) Boussard et al. demonstrate habituation in plasmodia, some biochemical process might be altered in a nonlinear way by repeated ligh exposure that would alter patterns of plasmodial morphogenesis much like those hypothesized to play a role in habituation. The *Physarum* light exposure-response may not be stable over time. Light exposure may change the concentration of biochemical solutes in the *Physarum* cytoplasm, which may have complex effects on morphogenesis. Following up on this work we plan on observing *Physarum* in a differentially illuminated environment. When one half of the plate is shaded, a photoavoidant morphological response can be measured by simply counting pixels on the halves of the plate. We can quantify photoavoidance directly. Like fungitaxis I do not expect all plasmodia to engage in photomorphogenesis the same way. Previously mentioned work has described *Physarum*'s capacity for habituation. As *Physarum* habituates, repellent stimuli lose their effect. This may be true with light exposure as well. By testing for photoavoidance in a half-illuminated dish we may be able to quantify the temporal dynamics of *Physarum* photohabituation. If these experiments demonstrate that photoavoidance exists and can be lost we may also find that habituation occurs at different rates across different plasmodia depending on their vascular architecture.

CHAPTER 5

Conclusion

5.1 IN SUMMARY

We were able to observe and characterize new biology in *Physarum polycephalum*. We were able to characterize the successional dynamics of *Physarum* and red yeast. While *Physarum* is most often cited for its capacity to approximate shortest paths between different food sources, there are other facets of its behavior that warrant attention. (Briard *et al.*, 2020) shows that *Physarum* slime sheath can provide an attractive or repulsive stimulus depending on the nutritive state of the depositing plasmodia (well-fed is attractive and starvation is repulsive). These aids to navigation help the *Physarum* more robustly navigate its environment in search of food. (Boussard *et al.*, 2019). We demonstrate that this deposited slime sheath may also act as an attractant for micro-organisms. This observation expands the importance of the slime sheath and betters our understanding of *Physarum* ecology. We are the first group to characterize the spatiotemporal dynamics of *Physarum* interactions with a micro-organism. Studying this successional dynamic we find an ecological function for *Physarum*'s slime sheath.

5.2 FUTURE WORK

Further research will better our understanding of *Physarum*'s habituative capacity to light exposure. While it is probably the case the photoavoidance response will be variable and may very well decay across time, we can directly measure photoavoidance and likewise the variance in the decay of this behavior. Photoavoidance quantified as the portion of plasmodial mass that is in the shaded half of the dish. Photoavoidance is not neccesarily a temporal behaviour. Photoavoidance may be measured across a single image, morphogenesis must be measured across several images of the same plasmodia.

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