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Analysing and Modelling the Oscillatory Pattern of Physarum Polycephalum with a Constraint Radius

Research Proposal for the Radboud Honours Academy FNWI

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1 Details

1.1 Details of Proposal

Title:	A novel information transfer model for the Physarum Polycephalum
Research field:	Unconventional computing; Biology; Physics;
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2 Preface

This research proposal was written during the academic year 2019-2020 as part of a special programme called the Honours Programme of the Science Faculty. This programme is part of the Radboud Honours Academy of the Radboud University in Nijmegen. In this programme up to 25 students from the Natural Sciences, Computing Science, Mathematics and Artificial Intelligence are chosen and split into groups of 4 to 5 people. The goal of each group is to write a research proposal in a topic of their choice. Due to the unique combination of the different fields the resulting topic is interdisciplinary, representing the different perspectives of each member of the group. Apart from the students each group is assigned a mentor that helps guiding the students while investigating different topics and ideas.

Overall this programme amounts to 12 European Credits on the duration of one year. During the first year each group needs to read academic papers, discuss them among each other, present their ideas to members of the programme and participate in a several skill training sessions which help the students to understand the secret behind a good research. In order to help the groups in developing their ideas and approaches the programme board organises a visit to a top university. During this visit each group meets with at least 2 professors, presents their progress and gets a feedback from an expert in the filed of their research. In this year we visited the University of Oxford where we got the chance to discuss with two experts, Dr. Mark Fricker and Dr. Alain Gorielywho, who wrote a few of the papers that were the inspiration of our topic.

Our group consists of students from 5 different disciplines Computing Science, Mathematics, Physics, Biology and Artificial Intelligence. Therefore we focused on investigating a unique organism, the Physarum Polycephalum, that has the ability to solve complicated problems. In order to deeper our knowledge in this field and come up with this research proposal we meet almost every week, most of the time together with our mentor Fleur Zeldenrust. In addition to those meetings and to all the literature that we have read, we also discussed with several experts both during the visit to Oxford and during the whole year. We would like to thank first of all our mentor Dr. Fleur Zeldenrust as well as Dr. Mark Fricker, Dr. Alain Goriely and his student research team, Dr. Karen Alim, and everyone else that provided help or feedback during the presentation sessions and the whole year.

3 Summary

3.1 Scientific summary

Physarum Polycephalum is a unicellular slime mold that is used a lot in the field of biological computing to solve computational network problems such as the travelling salesman problem. However, the underlying mechanisms of this seemingly intelligent behaviour remain unknown. Experimental data and models are used to investigate information transfer within the Physarum Polycephalum. A key feature is the oscillating behaviour of its plasmodium, which works through a cytoskeleton in the outer ectoplasm that contracts the inner endoplasm at regular intervals, pumping it to neighbouring areas. This displacing of endoplasm allows the P. Polycephalum to extend its body towards stimuli. We propose an experiment in which the tube radius of the P. Polycephalum is constrained while it reaches for a food source, due to a tunnel that is placed between the organism and the food source. Seen as natural environments can often be constrained, this could give insight into the P. Polycephalum's behaviour in such situations. In the proposed research, the radius of contraction, frequency of oscillation and spatial pattern of phase will be measured. The data will be fit with the leading bio-inspired models to verify their reliability, detect weaknesses and improve them in such a way that they give a better description of the P. Polycephalum. If the research proves fruitful, this data could eventually lead to new models and could be one of the building blocks in constructing novel bio-inspired algorithms.

3.2 Summary for the broad scientific public

Some of the biggest problems in computing science are the group of problems referred to as NP-complete problems, these are problems for which no efficient solution exists (as of yet) and which can only be solved by approximating the solution. Many approaches and approximations for these problems exist, but one specific research field shows great potential for creative approaches: biological computing. Most advances within this field have been made using computational models based on the single-celled slime mold Physarum Polycephalum. This organism is able to solve shortest path problems in nature and has been used to approximate, among others, solutions to the travelling salesman problem. This problem is as follows: given a set of cities and the roads between them, find the shortest path over the roads that starts in city X, passes through all the cities and comes back to city X. The main mechanisms being modelled are the search behaviour and the stimulus reaction or information transfer of the P. Polycephalum. It solves these problems by extending parts of its gelatinous-like body, referred to as tubes, towards a food source. These mechanisms are currently not well understood however, and modelling the Physarum Polycephalum to describe the mechanisms underlying its behaviour are not yet conclusive. The most well known hypotheses regarding information transfer in the P. Polycephalum all agree that they are grounded in its oscillatory behaviour.

We propose an experiment to gain insight into the Physarum Polycephalums oscillatory behaviour. In this experiment the tube radius of the P. Polycephalum is constrained while it reaches for a food source, due to a tunnel that is placed between it and the food source. Seen as natural environments can often be constrained, this could give insight into the P. Polycephalum behaviour in such situations. Furthermore the data collected by measuring the radius of contraction, frequency of oscillation and spatial pattern of phase can be used in creating new mathematical models describing the fundamental mechanisms of the P. Polycephalum. In the proposed research we will fit the leading bio-inspired models with the data, giving an idea of their general usefulness. If the research proves fruitful, this data could eventually lead to new models and could be one of the building blocks in constructing novel bio-inspired algorithms, with the grand aim of solving NP-hard problems.

4 Introduction

In the past researchers have used the Physarum Polycephalum in order to solve mazes [1], find the shortest path between different vertices by placing food sources on them [2], and provide approximations to several NP-hard and NP-complete problems, one of them being the famous travelling salesman problem [3]. By solving those problems, the Physarum Polycephalum shows an apparent capability of high level decision making, more commonly associated with life-forms that posses a nervous system [4]. How can the Physarum Polycephalum solve these seemingly "intelligent" problems, without a nervous system? It is tempting to believe that a single celled organism such as the Physarum Polycephalum can think for itself, that it can learn and has an intelligence reminiscent of that of humans. Many professional and hobbyist scientists fall for this temptation. We, as the authors of this research proposal, must admit that headlines such as "How Brainless Slime Molds Redefine Intelligence" [5] did indeed tempt us.

It is important to be realistic however, the behaviour displayed by the Physarum Polycephalum is not intelligence in the same sense as you and I are intelligent. The organism does not have a nervous system, but rather an externalized spatial intelligence. This means that the behaviour of the P. Polycephalum has no internal trigger, but is completely reactive to the environment. The slime molds' behaviour emerges from its morphological characteristics [4]. Nevertheless this organism demonstrates an ability to overcome obstacles that humans can learn from. By investigating the Physarum Polycephalum we might learn how to (better) solve difficult computational problems, like the aforementioned NP-hard and NP-complete problems. This is due to the fact that a mathematical model of the Physarum Polycephalum's behaviour can introduce a novel approach to solving problems. In the last decade many such bio-inspired mathematical models and algorithms have been created [6]. This started mainly because of the paper "A mathematical model for adaptive transport network in path finding by true slime mold" [2], but much research in slime mold path finding had been done even before that [1, 7]. Because bio-inspired algorithms are intended to solve computational problems, they sometimes take liberties with how accurately their models explain the P. Polycephalum's behaviour. Moreover, in those algorithms usually the researchers use only the properties of the Physarum Polycephalum that are beneficial for the respective problem [2, 8], hence the algorithms do not fully resemble how the real organism makes its decisions while solving those problems.
Models that more accurately portray the real slime mold have to be made to gain a better understanding of the P. Polycephalum's behaviour. The discovery of the principles underlying the P. Polycephalum's behaviour could then make way for more bio-inspired algorithms. Yet with all the research that has been done, researchers are still in the dark about the precise mechanisms behind the slime mold's intelligence. Accurately modelling these mechanisms could help in explaining the similar seemingly intelligent behaviour of many 1000's of organisms [9].
In this project we aim to further investigate the Physarum Polycephalum and collect data by conducting an experiment on the Physarum Polycephalum. In the experiment we will check how putting a constraint on the radius of the tube will affect the oscillatory behaviour of the Physarum Polycephalum while trying to reach for a food source. This could give insight into how the Physarum Polycephalum adapts to space-constraint environments and how oscillatory patterns in the P. Polycephalum adapt in general. The extracted data will be used to fit existing bio-inspired models. These models, while not a complete accurate depiction of the P. Polycephalum, can give insight into the accuracy of this data, before trying to construct a model from scratch to describe the P. Polycepalums behaviour.
We hope that this research will help to gain a better understanding of the behaviour of the Physarum Polycephalum and will be one of the building blocks in creating mathematical models that cover a larger range of the properties of the Physarum Polycephalum aimed at explaining its 'intelligent' behaviour.

5 Scientific Background

Physarum Polycephalum research is an active field of science both in terms of fundamental research [10, 11] and computational application for example in bio-based sensors [12] or in solving network problems [2, 8]. Therefore, it is important to clarify what is known about Physarum Polycephalum and what is still unknown. This section first gives an overview of the properties of the Physarum that are important for the proposed experiment and explains which open questions are to be answered by the project. Because the project does not only concern the fundamental Physarum Polycephalum research but also has as an ultimate goal the computational application of the gained knowledge, the second part of this section provides an overview of the basics of P. Polycephalum modelling and computing.

5.1 Physarum Polycephalum

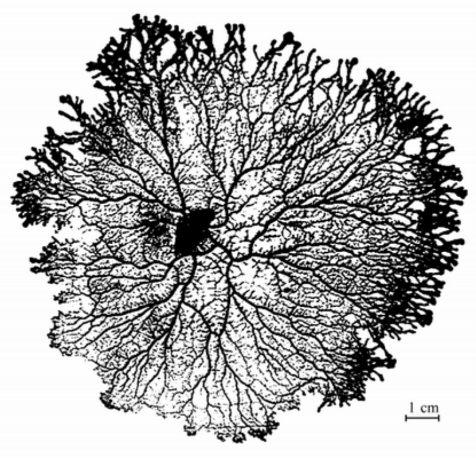

Figure 1: Full Physarum Polycephalum network. Figure reprinted from [11]

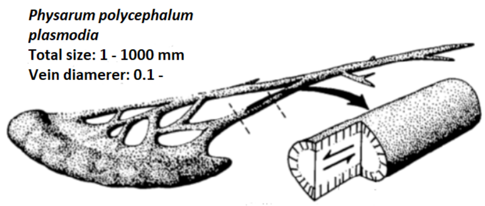

Figure 2: Diagram of a single tube with a fan-like structure at the front. The tube at the left side gives insight into the inner structure of the tube consisting of the outer ectoplasm (striped part) and the inner flowing endoplasm (white area). The arrows in the endoplasm symbolise the shuttle streaming. Figure reprinted from [13].

Physarum Polycephalum is an acellular, multinucleated slime mold species. They begin their life cycle as haploid amoeba-like cells, which will fuse into diploid zygotes and develop into a multi-nuclei single cell. This structure is called "plasmodium", which is visible for the human eyes as a large yellow slime structure and can extend up to one square meter. The plasmodium contains freely moving cell organelles such as multiple diploid nuclei, mitochondria, food vacuoles, etc. which are all enclosed by the plasma membrane.
P. Polycephalum does not possess many permanent structures, because the amoeboid cell is continuously changing its shape in adaptation to environmental factors and life cycle requirements [14]. With the spectacular ability to expand the plasmodium, P. Polycephalum shows complex patterns of locomotion and behaviour. Physarum Polycephalum locomotion happens via network expansion. Such a network is shown in figure 1. The main building blocks of the network are tubes (veins) which part into fan-like structures in the front area, this can be seen for a single tube in figure 2. A single tube consists of fluid endoplasm that is enclosed by an outer gel-like ectoplasm. Furthermore, the ectoplasm contains an actomyosin network, of which the building blocks are actin and myosin proteins. In cell biology, actomyosin is the basis of cell migration. In human physiology, the muscle movements: contraction and relaxation are driven by the interaction between actin and myosin. The two striking mechanisms of a tube accompanying Physarum Polycephalum locomotion are rhythmic contractions of the ectoplasm and endoplasmic shuttle streaming [11]. The contractions which have a period of about 60s-120s are controlled by the actomyosin protein network [15] and cause the flow of the endoplasm to rhythmically change its direction (shuttle streaming) [13].

Physarum Polycephalum as a coupled oscillator system

As previously mentioned, the form of the network is influenced by environmental factors. Adaptation to the environment requires transfer of information. The exact mechanism of how information is transferred via P. Polycephalum's body is unknown [10], but oscillations seem to play a key role [11]. Currently there are three main hypotheses for explaining information transfer in Physarum Polycephalum: information might be transferred via 1) elastic waves [16], 2) via en advected molecular stimulus [17, 18, 10] or 3) via electrical impulses [19]. All of those components manifest their nature through oscillations. Synchronous oscillations of the membrane potential [11] and of the concentrations of Ca^{2+} [20, 21], H^{+} [22], ATP [23] and other molecular stimuli have been observed. Remarkable is that electrical and chemical oscillations always arise together with mechanical oscillations, but the inverse is not true [11]. Due to this fact, mechanical waves are the main focus of this research. Although the question whether all these oscillations are ruled by a single underlying mechanism and what this mechanism is remains open, the model of a coupled oscillator is well established [11]. This means that the oscillatory is well distributed throughout the entire organism and the oscillatory behaviour at one particular point in the organism is directly connected to the oscillatory patterns of its neighbouring regions. Experimental evidence for this does exist: First, if a single strand is cut out from the main body and placed on a surface it will start to contract after $10-20$min. That a single strand is able to contract means that all properties of the oscillator are contained in this strand, this holds for any arbitrary strand, therefore the oscillator is well distributed throughout the entire Physarum Polycephalum [24]. Second, coupling is indicated by the Physarum Polycephalums coordinated reaction to external stimuli, for instance exposing one local part of the Physarum to a food source can cause the entire organism to move towards the food source [25]. Therefore, the local contractions in Physarum Polycephalum are determined both by the contraction of the neighbouring regions and the environment. Before elaborating further on the various contraction patterns that have been observed in the P. Polycephalum it is useful to introduce terms that enable a mathematical description of contraction:
In general, for describing contraction waves the terms amplitude, period and phase are used. The amplitude is the maximum radial derivation from the mean radius. The period is the duration of one full contraction cycle. The inverse of the period is the frequency which corresponds the number of oscillations per unit time. The phase of a wave is a measure of how much of the period has passed by [26]. In the case of a contraction wave it is useful to define the phase "as the contraction cycle elapsed relative to the last maximum" [27].

Oscillation and contraction patterns

The plasmodium of the Physarum Polycephalum shows various types of oscillation patterns including undisturbed propagation of contraction waves, collision, splitting and annihilation of contraction waves and the formation of spiral contraction waves [28]. In general, the complexity of the oscillations depends on the size of the Physarum Polycephalum and environmental factors [24]. But preferably, P. Polycephalum tries to adjust the phase of contraction such that there is only one wavelength across the entire organism [10].

Response to external stimuli

External stimuli such as a food source influence the contraction pattern of the P. Polycephalum because it functions based on reactive navigation via chemotaxis: when a plasmodium encounters a chemoattractant, it will bind to a specific receptor on the plasma membrane, causing an increase of the oscillation frequency in the contacted area. Hence, the plasmodium expands and then moves towards this area of higher oscillation frequency. In contrast, the plasmodium moves away from repellents, which locally decrease the frequency [25]. Besides the frequency, the amplitude also increases at the part of the Physarum close to the chemoattractant. This generates an amplitude front that propagates through the organism [10].
A food source is a chemoattractant. Thus the food source in the experiment should cause an increase in the contraction frequency and amplitude. An example of a repellent is UV and short wavelength visible light [29]. Other factors that can influence the contraction pattern and that are important for the experimental set up are the temperature[30] and humidity of the atmosphere [30]. For instance

the higher the temperature the higher the frequency of contraction. A temperature difference of $4 \circ C$ can already cause a rise in frequency by $3Hz$ [30]. Given all these factors that influence the oscillation pattern, it is important to ensure that the environment is kept as constant as possible in the experiments.

Constraining the contraction amplitude

To the best of our knowledge, no research has been done so far investigating the oscillation pattern under a constrained tube radius. Nonetheless, observing the frequency under a constrained amplitude can contribute to the understanding of the underlying oscillation mechanism. Additionally, the reaction and adaption of the whole organism according to the constraint gives new insights into how the Physarum communicates across its entire body. Moreover, seen as the known information transfer hypotheses all have a basis in oscillations, a constraint on the oscillations could affect the information transfer. When information transfer is affected this could possibly be seen in the behaviour of the P. Polycephalum. Finally, there are several applications that would profit from knowing the exact oscillation pattern of Physarum Polycephalum with a constrained radius such as the implementation of Physarum Polycephalum into sensors or other computational devices with limited space. Another field of application is solving network optimisation problems with different constraints on different paths.

5.2 Physarum Polycephalum modelling

When talking about modelling in terms of biological organisms in general, and Physarum Polycephalum in particular, researchers usually refer to creating a model that will focus on the Physarum Polycephalum's foraging behaviour in order to better understand its core features and the underlying mechanisms [6]. These mechanisms can later be used in the field of bio-inspired computation in order to solve complex computational problems.
Behaviour explanation algorithms are the most varied. As the mechanisms underlying the P. Polycephalum's behaviour are not fully understood [10, 11], one can imagine that this area is where most experimentation takes place. Because there exists a plethora of models already, this proposal will focus on testing these existing models against the generated data, rather than focus on developing new models. While these are not always a complete accurate depiction of the P. Polycephalum, they can give insight into it's behaviour. By fitting algorithms with the data collected during the proposed experiments the data can be tested on accuracy, before trying to create a completely novel model out of it.
There are 4 main bio-inspired models of the Physarum Polycephalum in the literature: models based on morphology, gradient-based model, models based on current reinforcement dynamics and agent-based models[6]. For each one of these, an explanation of the main idea follows in the next section.

Models based on morphology

First, we start with the models based on morphology, the goal of this model is to try and simulation the behaviour of the Physarum Polycephalum in a maze that is based on grid of cells. The idea is as follows, when the Physarum Polycephalum senses a food source, the protoplasm extends towards it, thereby shifting the body mass of the P. Polycephalum away from other regions, towards the protrusion. In order to better explain the model, let's consider a grid with cells as shown in Figure 3. In that figure A represents the real experiment, B is the general set up of the cells and C is the an example for a possible path choose by the slime mold in one of the execution that we are going to explain.In this model we split the cells into 2 categories: internal and external. External cells are cells that the Physarum Polycephalum can start from, represented by a white cell in Figure 3. The internal cells are split into 3 types of cells, 'active zones' which are the cells that represent the position of the food source (represented as grey cells in Figure 3), 'path cells' which are the cells related to the extension of the organism (represented as a blue cell in Figure 3) and 'active cell' also called Bubble which represent the emerging protoplasm that is generated within a grid of possible routes (represented as a black cell in Figure 3) [31]. The cell that is responsible for the movement of the P. Polycephalum is the 'active cell' which have 3 actions: generating, moving, and replacing. In the generation phase the P. Polycephalum is randomly placed on one external cell next to an active zone where it will start its movement (second image from part C). Then the moving phase starts where the cell will move to an 'active zone' cell changing the state of the cell from 'active zone' cell to 'path cell' repeatedly until there are no more 'active zone' cells in its neighbourhood (the 7th image of part C). Finally in the replacing phase, the cell where the above procedure terminated will change its state to external cell and the cell that was picked at first will change it state into 'active zone' cell (as can be seen in the last image of part C). Those 3 phases are repeated until we get a route that connects all the active zones. The final route which will consist of only 'path cells', approximates an efficient network that should simulate the behaviour of the Physarum Polycephalum according to this model. [31][32].

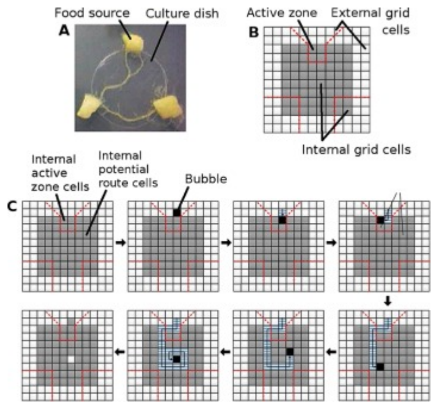

Figure 3: Model based on morphology. Figure reprinted from [6]

Gradient-based models

Gradient-based models exploit the fact that the Physarum Polycephalum can move towards different food sources around it. Unlike the previous model, which was more like an algorithm, this model is based on mathematical equations. In this case we consider a 2 dimensional graph such that each point has two coordinates x and y. Each food source attracts the Physarum Polycephalum based on the following: food source point of origin $O_i = (x_i, y_i)$, intensity φ_i, major a_i and minor b_i semi-axes. We calculate the attraction value based on the coordinates of the Physarum Polycephalum P = (x,y) as follows $f_i(P) = \varphi_i \exp[-d(O_i, P)]$ where $d(O_i, P) = \sqrt{((x_i - x)/a_i)^2 + ((y_i - y)/b_i)^2}$. So the attraction of multiple sources is a superposition of individual attraction, $f(P) = \sum_i f_i(P)$. Finally the unit gradient vector of field f at point P is defined by:
$N(P) = (\partial_x f(P), \partial_y F(P))/||N(P)||$ where $||\cdot||$ is the vector norm. The plasmodium at point P_t at time t will move to P_{t+1} at moment t+1 based on $P_{t+1} = P_t + \delta N(P)$ where δ is a parameter controlling the speed of movement [6].

In the simplest case where all food source intensities are the same, Physarum's plasmodium will extend towards the closest food source until it has reached it. Then the attraction of this food source is removed from $f(P)$ and the procedure will be repeated until all desired points are connected. This eventually will result in a route connecting all desired points that should simulate the behaviour of the Physarum Polycephalum according to this model.

Models based on current reinforcement dynamics

Models based on current reinforcement dynamics simulate the Physarum Polycephalum's behaviour by modelling the increase and decrease in radius of tubes due to flow through the system. The name for the type of these models comes from them exploiting the feedback loop where: 1. Short tubes carry more flow than longer tubes, the larger flow forces them to grow in radius. 2. The increased radius allows these tubes to carry more flow. The increase in tube radius is thus a self-reinforcing

system in this model. The total in and out flow of all the tubes in the system together remains constant to conserve the Physarum Polycephalum's mass. This means other tubes decrease in flow and thus in radius when a tube increases in flow.
This model can in general be described as a graph where e_{ij} is a tube between nodes N_i and N_j, D_{ij} measures the conductivity through e_{ij}, this can be used to measure the radius. Q_{ij} is the flux through e_{ij} and L_{ij} is the length of e_{ij}. The following formula's are iteratively applied in the model to form the Physarum's behaviour through time. The flux Q is described by:

$$(1)\ Q_{ij} = \frac{D_{ij}}{L_{ij}}(p_i - p_j)$$

which determines the relationship between the flux and the conductivity, D_{ij}. p_i and p_j represent the pressure at N_i and N_j respectively. Conservation of mass is modelled as:

$$(2) \sum_i Q_{ij} = \begin{cases} -I_0, & if j = in \\ I_0 & if j = out \\ 0 & \text{otherwise} \end{cases}$$

where -I_0 is the net inflow of flux and I_0 the net outflow.

Finally, the change in D is described by:

$$(3)\ \frac{d}{dt} D_{ij} = f(|Q_{ij}|) - rD_{ij}$$

where f is some function which is different depending on the problem the model is applied to. When D_{ij} converges to 0, the corresponding tube e_{ij} disappears, to simulate the removal of tubes. A maximum value means the tube stays. When this models is used for solving shortest path problems, all tubes not in the shortest path will converge to 0 [6], such that a single shortest path eventually remains.

Agent-based models

Agent-based models approach the Physarum Polycephalum from the angle of emergent behaviour. Meaning there are many small components referred to as "agents" that together form the slime mold. Each agent has the same set of rules for their interaction with the environment and other agents in the system. The system is run using time steps, where at each time step an agent can take a certain action. The general idea is that when an agent detects a food source, they take an action which directs them towards the source and they leave a trail which attracts other agents. From the interaction of the agents emerges the behaviour of a Physarum Polycephalum throughout a certain time frame when interacting with food sources. [6].

6 Research Question

After our preliminary literature study, we had noticed, as specified earlier, that the way in which the signal propagation take place in the Physarum Polycephalum is still unknown [10, 11]. While there are quite a few hypothesis including elastic waves, advected molecular stimuli and electrical impulses [10] it is still not known which one of them is the correct one or whether it is a combination of them. At first, we wanted to try and face this issue, trying to answer the question of which one of them gives the most accurate representation but we found this question to be too elaborate for one research proposal and would be better served by multiple research proposals. Therefore, we decided to focus on only one of those methods, the elastic waves, and construct a novel experiment. Since Physarum Polycephalum research usually includes both experiments and models [10, 27] the research question is two parted:

In what way will the oscillatory behaviour of the Physarum Polycephalum change when its tube radius is constrained when reaching for a food source and how can this be described by existing models?

The hypothesis is, that the oscillatory behaviour will be regular while the P. Polycephalum is extending its tube through the tunnel and will become irregular as it reaches the food source. A regular oscillation pattern means a standing-wave-like pattern with a fixed contraction amplitude and frequency, while irregular refers to contractions with constantly changing amplitudes, frequencies and phases. After a limited time the P. Polycephalum will return to a regular oscillation pattern.

While inside of the tunnel, the P. Polycephalum can perform its normal searching behaviour, but just has less space to search. The process of moving towards the food source would normally cause the tube closest to the food source to extend towards it. When the food source has been reached the radius and the contraction frequency increase of the connected tube increase, while tubes further away from the a food source decrease in radius. Now that the tube radius is constrained, the mechanism for expanding the tube size still will be triggered, but it will not be possible to increase the radius of the tube, causing a conflict between the signals for radius increase and the allowed radius increase from the environment. This conflict induces irregular patterns in the oscillatory behaviour. Ultimately the Physarum Physarum will adapt to the environmental constraints and its regular oscillatory behaviour will return.

Our research question and hypothesis naturally leads to a few sub-questions that needed to be answered. Thus raising the experiment that need to be conducted in order to analyse these questions. Those sub-questions are:

1. How does changing the diameter of the tunnel affect the pattern of oscillation change during the experiment?
2. How will the oscillations differ between the part of the Physarum Polycephalum which is inside the tunnel and the part that is outside the tunnel?
3. To what extent will the known models of the Physarum Polycephalum represent the behaviour of the Physarum Polycephalum in this experiment?

7 Research Outline

7.1 Setup

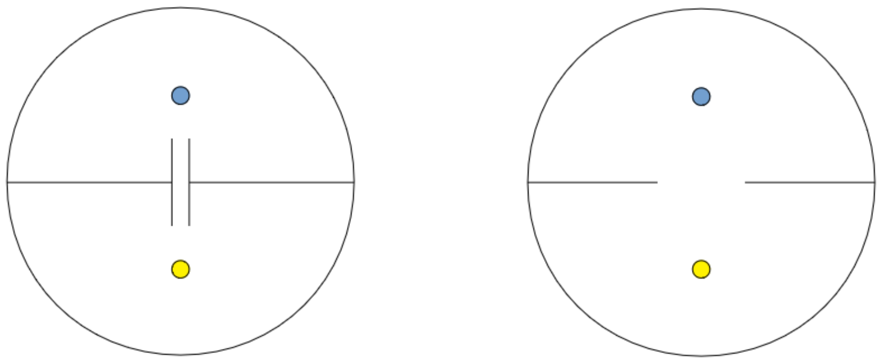

Figure 4: The set up: On the left the Petri dish with a tunnel. On the right the set up for the positive control. The yellow point marks the starting point of the Physarum. The blue point represents the food souce.

The goal of the experiment is to investigate how a constraint on plasmodial tube's radius will change the contraction pattern of the Physarum Polycephalum. Figure 4 schematically shows the setup. The setup is described as follows: A Physarum Polycephalum's plasmodium is placed on one end of the tunnel. On the other end of the tunnel, an oat flake will be placed. The oat flake acts as an attractant and thus makes the slime mold pass through the tunnel. The tunnel has a diameter that constrains the plasmodial tube's radius of the P. Polycephalum as it passes through it. The tunnel length is constant over experiments, but the diameter of the tunnel is manipulated and is therefore the variable quantity over various experimental runs.
To characterise the oscillatory behaviour of the Physarum Polycephalum the following properties will be measured:

1. Radius of contraction
2. Period of contraction
3. Spatial pattern of phase

In the following sections, the materials are first described, and then the measurement procedure will be explained. To avoid the influence of characteristics of an individual slime mold or an individual set up the experiment must be conducted with multiple Physarum Polycephalum.

Physarum Polycephalum

Three different P. Polycephalum will partake in the experiment with 3 different tunnel sizes: 50um, 40um and 30um. For the experiments the P. Polycephalum will have the same hunger level by having a consistent culturing of P. Polycephalum.

7.1.1 Environment

Twelve agar Petri dishes will be used in total in this experiment. Three out of twelve will not be constructed with tunnels, which will act as a positive control where the plasmodium is under no constraint. For the tunnel setup, each tunnel size will be prepared in three agar Petri dishes. Moreover, every Petri dish will be contrusted with a horizontal wall in order to make sure no network will be formed around the tunnel. Plates with tunnel condition will have a tunnel in penetrating through the wall. Positive control plates will have a hole of 2cm in the middle of the wall.

7.1.2 Food source

The food source are oat flakes, as this is a food source that is most often used in the literature. For each iteration of the experiment the same quantity and brand (e.g. Quaker Oats Company) will be used.

7.1.3 Tunnel

The tunnels are made of crystal glass. Every tunnel has the same length - 50mm, but tunnels with different radii are used - 50um, 40um, 30um. The diameter is the only variable that is varied for the tunnels. Every individual will partake in four different experiments; a control experiment without a tunnel and three with the tunnels of each radius.

7.2 Measurement

The spatiotemporal development of a periodically contracting tube is shaped by four parameters: the spatial pattern of phase, the baseline radius of the tube, and the amplitude and period of the contractions [27]. In this experiment, different constraint extents will be put on the maximum growing tube radii. Consequently, we want to observe the P. Polycephalum's spatiotemporal development under this radius constraint condition by examining three out of four underlying parameters: the spatial pattern of phase, the baseline radius of contraction, and the period of the contractions.

7.2.1 Physarum Polycephalum culturing

Before conducting the experiment, P. Polycephalum will be grown on an agar plate without nutrients and fed daily with oat flakes. By doing this, we can make sure that the organisms are always in vegetative stage. The vegetative stage is characterized by a large multi-nucleate plasmodium which behaves like a single cell [33], which is our desired form of the P. Polycephalum. After 18-24h, P. Polycephalum will form a tubule network of plasmodia [34]. Newly colonized oat flakes will be transferred to new Petri plates, including a normal agar plate and plates with the tunnels of different radii. The normal agar plate will be used as a positive control, in which the plasmodia can explore the environment and expand without any constraint. Plasmodial tubules of around 2.5cm will be cut from the already cultured P. Polycephalum, and moved to the new agar plates. The plasmodium will then explore the new surface and form a new network. With the tunnel-constructed agar plate, we will illuminate the outside surroundings of the tunnel with blue light (460nm). At this intensity, blue light will induce plasmodial fragmentation [35]. Hence, P. Polycephalum cannot migrate along the tunnel from the outside. Before imaging, the recessive tissues are scraped away so that the entire network will fit within the field of the view [10].
This protocol will be repeated for every plasmodium in each of the four conditions: no tunnel, tunnel with radius of 50um, 40um, and 30um. In total, twelve plasmodial contraction processes will be observed.

7.2.2 Imaging

The suitable microscopic technique for observing and recording radii of plasmodial tubules during contraction is a stereomicroscope, together with a high-resolution time-lapse camera. We will use magnifications between 1.0x and 10.0x for the microscope. The total duration of the experiment is expected to be 2 to 3 hours, depending on how fast the P. Polycephalum navigates to the oat flakes. Every dish will be illuminated a light source from below. A long-pass filter of 610nm will be put underneath the plates, right on top of the light source. With this filter, only light with a wavelength longer than 610nm can pass through, which is a safe wavelength for the P. Polycephalum. The illuminated light from below will be detected by the microscope and the camera, making the plasmodia distinguishable from the background. The experiment will be run for two to three hours. Images will be taken every 6 seconds.

7.2.3 Measuring baseline radius of contraction

The radii will be measured by a computer vision script written in MATLAB. For each captured image, the script will identify the plasmodial tube edges. The script positions a circle at the edges and adjust the diameter to match the thickness of the tube. This will be repeated at 50 equally spaced locations.
The final baseline radius of contraction will be determined by taking the average of ten largest baseline radii.

7.2.4 Measuring period of contraction

In order to identify the period of contraction and the pattern of phase, the transmitted light intensity during the experiment will be recorded. The transmitted light intensity will differ between contraction and relaxation: higher and lower pixel intensity represents contraction and relaxation, respectively. When the P. Polycephalum is contracting, plasmodial matters are more concentrated at the contraction point, therefore, the pixel intensity will be higher. By this properties, the oscillating cross-sectional contractions of a tube directly modulate the intensity of transmitted light [27]. For each point in the P. Polycephalum network the pixel intensity against the time can be plotted. This gives a periodic graph as shown in figure 5.

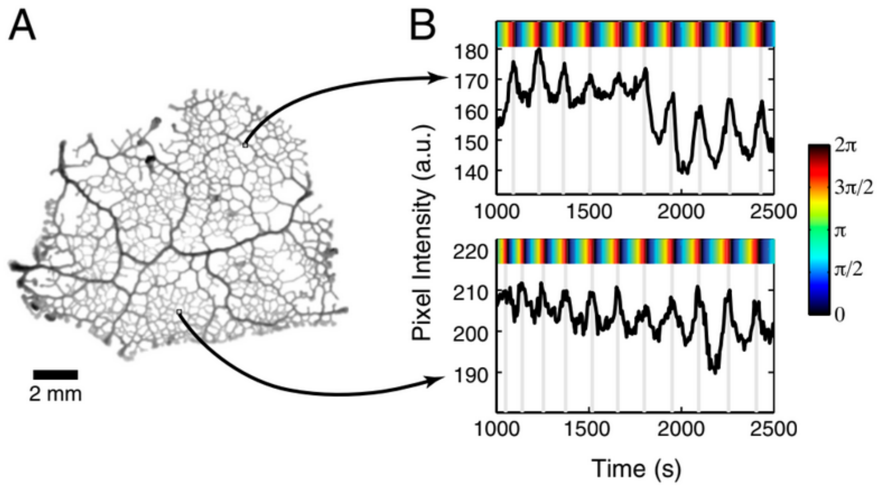

Figure 5: Visualising the measurement procedure: A is a Bright-field image of the Physarum Polycephalum network. B shows two examples of pixel-intensity-against-time plot that can be made for every position. Figure reprinted from [27].

The period of contraction is than defined as the time from one intensity maximum to the next intensity maximum and can directly be read of from the pixel-intensity-against-time-plot.

7.2.5 Measuring spatial pattern of phase

The phase can be calculated based on the same plot. The phase varies with the same periodicity as the contraction. In other words the phase is scaled in such a way that locally the phase variation from one maximum radius to the next maximum, thus during one period, is equal to 2π. One maximum to the next minimum corresponds to a phase of π. Based on the previously definition of phase "as the contraction cycle elapsed relative to the last maximum"[27]. The phase can be calculated from the pixel-intensity-against-time-plot.
Applying the above mentioned method to every part of the P. Polycephalum for every point in time gives an overview of the evolution of the contraction pattern of the whole Physarum Polycephalum. An algorithm will be implemented in order to extract the amplitude and the phase from the light intensity.

7.3 Model development and conclusions

After having collected the data, we will fit a variety of models to the data, using

1. Models based on morphology [31, 32]
2. The gradient-based model [6]
3. Reinforcement Dynamics [6]
4. Agent-based models [6]

All of these have been used previously in the scientific literature [6, 31, 32, 34], the models need to be adapted to the described situation including the tunnel and fitted to the data. Finally, we will compare these models in terms of a number of metrics, such as the convergence speed, similarity to ground truth and robustness to determine the relative strengths and weaknesses of the different models.

7.3.1 Convergence speed

For each developed model, we will define some time unit so the signal frequency will, on average, match that of the real P. Polycephalum. Then, we will measure how quickly each model tends to its final stable state, and so its convergence speed. In general, a greater convergence speed suggests that the model is more robust and models that exhibit such behaviour will therefore be preferred.

7.3.2 Similarity to ground truth

For each model, we will compare the distribution of the oscillation frequency, amplitude and phase to the data collected for similar setups. Models that behave similarly to the real P. Polycephalum are more likely to share other desired properties that the P. Polycephalum might hold, and could also prove useful in the further investigation of P. Polycephalum behaviour, and so using this metric we will be able to compare models in terms of these properties.

7.3.3 Robustness

A more robust model is better when adapted to various problems, and comparing the various models in terms of robustness is, therefore, a necessary step before the adaptation of the models to other problems. There is no universal way to judge the models on robustness, so instead we will judge each method subjectively weighing its design and performance, and in the end will compare the models based on this judgement.

8 Knowledge utilization

The outcome of this research can be divided into the **description of Physarum Polycephalum's contraction waves with a constraint amplitude** and **comparison of different Physarum Polycephalum models**. Therefore, both scientists that do fundamental research about P. Polycephalum and researchers working in the field of unconventional computing will profit from the results.

8.1 Fundamental Physarum Polycephalum research

Researchers working on fundamental Physarum Polycephalum research are interested in understanding the processes going on insight P. Polycephalum. As mentioned in the scientific background (section 5.1) the mechanism that drives the oscillations of the P. Polycephalum is not fully understood yet [11]. The data might lead to a clarification of understanding the underlying oscillator of the Physarum Polycephalum in particular the relationship between the amplitude and the frequency of oscillation. In this context the experimental result might also be the bases for further experiments and measurements on P. Polycephalum constraints. In particular, measurements of the concentration of different chemical stimuli as well as the membrane potential will lead to more insight in how information is transferred in P. Polycephalum. This idea will further be exploit in the Future Prospects part (section 12).
Concerning the models, the comparison of different models can verify the reliability of each model, detect weaknesses and can led to an improvement of the models in such a way that they give a better description of the Physarum.

8.2 Unconventional computing

Unconventional and in particular nature-inspired computing is an emerging field of science including quantum computation, membrane computing and artificial immune systems. The main work in this field is theory based, due to the technical difficulties and high costs connected with building unconventional computers. Because Physarum Polycephalum can be easily cultivated and experimented with it provides a unique opportunity to actual realise bio-inspired computers and is therefore, a good starting point to put theory into practice [36]. Physarum Polycephalums computation is usually done by making use of the morphological adaption of P. Polycephalums network structure, using the wave propagation of information through the protoplasmic network or investigating the oscillations arising in the plasmodium [37].
Indeed Physarum polycephalum has already been implemented into tractile bristles sensors [38] and was used to accomplish computational tasks such as maze-solving [1], calculation of efficient networks [39] or construction of logical gates [40]. The results are promising, but most of the applications are done in laboratory in an experimental set-up, to build a real Physarum machine more knowledge about the computation behaviour of the P. Polycephalum and about how Physarum Polycephalum, might be integrated into technical devices is necessary.
The data from this experiment, in particular knowing the oscillatory behaviour of the Physarum Polycephalum under a constraint, can be applied to integrate Physarum Polycephalum into computational devices with limited space. Furthermore, the experimental results might be inspiring for new Physarum Polycephalum based solutions of network-optimization problems such as networks with different path capacities.

8.3 Outreach

As indicated above research groups doing fundamental Physarum Polycephalum research as well as people working in the field of unconventional computing will benefit from the results. To reach both groups it should be considered to publish the results in journals from the field of biophysics e.g. "Cell Research" and computing science and to present the results at different conferences. Also interdisciplinary journals with a broad audience such as "Nature" will be considered. Another two journals that we can use in order to outreach our paper are "IEEE Transactions on Evolutionary Computation" and "Applied soft computing" which publish papers that focus on evolutionary computation and related areas such as nature-inspired algorithms. Thus, we can reach researchers that are interested both in biology and computer science, especially in bio-inspired computation approaches. In terms of conferences the "International Conference on Unconventional Computation and Natural Computation" might be interesting.

9 Time schedule

	Year 1	Year 2	Year 3
Laboratory experiments			
Data analysis			
Modelling			
Writing			

Figure 6: Work distribution for one full-time Biophysics PhD students
Please note:

1. Laboratory experiments include the culturing and measurements with the P. Polycephalum.
2. The data analysis requires implementing algorithms to get the required quantities (radius, period and phase).
3. Modelling means to align the models with the constraint and running the test the models on the obtained data

10 Cost estimation

Type	Amount per unit	Units	Total
PhD students in Biophysics	30 euros per hour	4160 hours	124800 euros
Microscope	100 euros per hour	60 hours	6000 euros
Camera equipment	100 euros per hour	60 hours	6000 euros
P. Polycephalum	20 euros per unit	6 units	120 euros
Tunnels for each P. Polycephalum	30 euros per unit	3 units	90 euros
Total (lower bound)			137110 euros
Total (+10%)			**148821 euros**

11 Risk Assessment

First of all, the culturing of the Physarum Polycephalum needs to be very precise. Its sensitivity is due to its ability to switch to a new life form, triggered by the environment (Figure 7). The feeding amount, light and moist condition needs to be examined carefully. We will order three extra Physarum Polycephalum in case the first treatment groups fail.

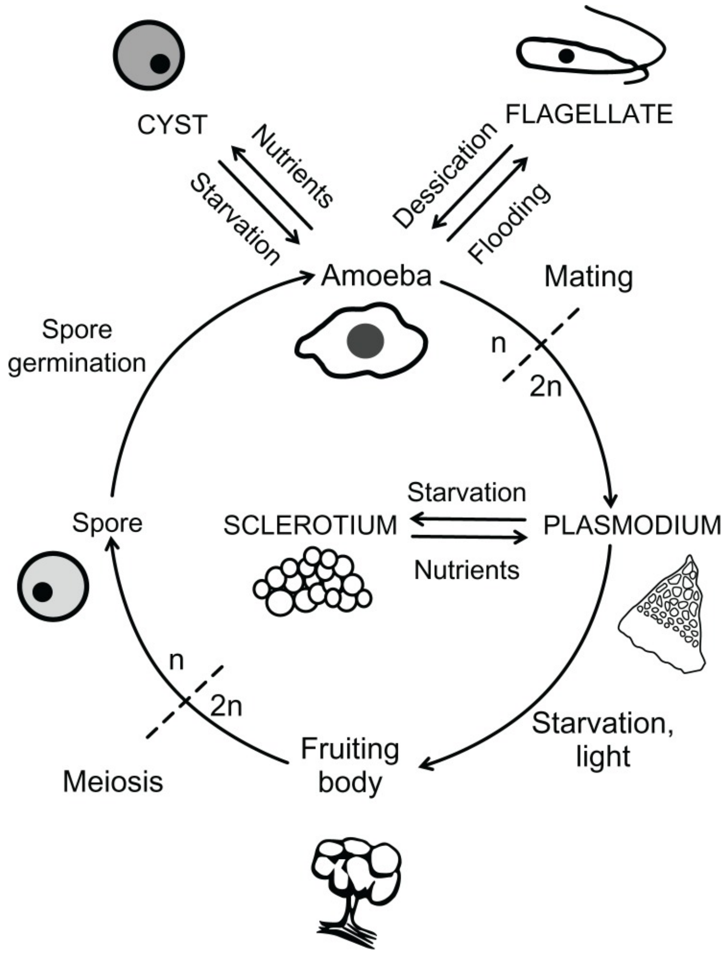

Figure 7: Life cycle of Physarum Polycephalum. Figure reprinted from [41]

Moving on to the setup, the tunnel needs to be completely sterile. Once again, P. Polycephalum is a very sensitive organism, hence, any substance can act as an attractant and can affect the oscillating process of the plasmodium. Therefore, we propose treating the crystal material with ethanol 97% and let it dry next to a blue flame. Moreover, the inhibiting light also is not allowed to scatter away from the illuminating point or penetrate inside the tunnel. That is why the crystal material will be created with a bypass filter that does not allow blue light at 460nm.

In case the inhibiting light source cannot be controlled properly, we intend to use inhibiting chemical substances and introduce them to the surface which we do not want the plasmodia to migrate through.

The experimental setup can also hold a possibility that the tunnel radius is too small for the P. Polycephalum to pass through. We will try to solve this by adding double the number of oat flakes in order to attract the P. Polycephalum more. If this solution still does not work out, we can come up with a minimum required plasmodial tube size that the P. Physarum has to reach in order to contract and relax.

The properties of the each P. Polycephalum within the species. Large deviations in radius of contraction, period of contraction, and spatial pattern of phase among organisms might occur. If this happens, outliers will be removed. Subsequently, we will use GPower, a statistical tool to calculate the new sample size to compensate for the deviations. As a result, we might need to order new P. Polycephalum.

Moreover, we also think about the possibility that the P. Polycephalum will not change its oscillation pattern even under radius constraint. In Karen Alim's study about peristalsis pattern of fluid flow within the P. Polycephalum [27], a theory was proposed that peristaltic wave patterns result from the interplay of a local rule (minimizing the phase differences between neighboring vein segments) and a global constraint (mass conservation). Therefore, if the oscillation pattern does not change, the global constraint must have played a role in this. Hence, more scientific researches can focus on the mass conservation law in the organism.

Finally, it is possible that some of the models will prove to be unable to be fit do the situation, in which case we will leave that model out of consideration.

12 Future Prospects

Constraints on the tube radius could provide important insights into the understanding of the oscillatory behaviour of the P. Polycephalum. The most important insight would be about the information transfer within the P. Polycephalum. As previously mentioned there are three main hypotheses for information transfer: elastic waves, advected molecular stimuli and electrical signals [10]. Since this proposal focuses on the first one, measurements of the other two parameters might be conducted in order to investigate the role of each in information transfer.
The modifications that are made to the models to fit the constraint may be used as inspiration to derive models for other constrained situations. If the model proves to be an accurate representation of the real P. Polycephalum's behaviour, then the model could also generalize to organisms with similar oscillatory behaviour [9]. Finally, further research could be done into the application of the models produced by this study to various kinds of computational problems. The newly derived models can then be compared to already existing methods in terms of performance on said problem.

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