UNIVERSITY OF THE WEST OF ENGLAND

On the development of slime mould morphological, intracellular and heterotic computing devices.

by

Richard Mayne

A thesis submitted in partial fulfillment for the
degree of Doctor of Philosophy

in the
Faculty of the Environment and Technology
Department of Computer Science and Creative Technologies

June 2016

Declaration of Authorship

I, RICHARD MAYNE, declare that this thesis titled, 'On the development of slime mould morphological, intracellular and heterotic computing devices' and the work presented in it are my own. I confirm that:

- This work was done wholly in candidature for a research degree at this University.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: _____

Date: _____

UNIVERSITY OF THE WEST OF ENGLAND

Abstract

Faculty of the Environment and Technology
Department of Computer Science and Creative Technologies

Doctor of Philosophy

by Richard Mayne

The use of live biological substrates in the fabrication of unconventional computing (UC) devices is steadily transcending the barriers between science fiction and reality, but efforts in this direction are impeded by ethical considerations, the field's restrictively broad multidisciplinarity and our incomplete knowledge of fundamental biological processes. As such, very few functional prototypes of biological UC devices have been produced to date. This thesis aims to demonstrate the computational polymorphism and polyfunctionality of a chosen biological substrate — slime mould *Physarum polycephalum*, an arguably 'simple' single-celled organism — and how these properties can be harnessed to create laboratory experimental prototypes of functionally-useful biological UC prototypes. Computing devices utilising live slime mould as their key constituent element can be developed into a) heterotic, or hybrid devices, which are based on electrical recognition of slime mould behaviour via machine-organism interfaces, b) whole-organism-scale morphological processors, whose output is the organism's morphological adaptation to environmental stimuli (input) and c) intracellular processors wherein data are represented by energetic signalling events mediated by the cytoskeleton, a nano-scale protein network. It is demonstrated that each category of device is capable of implementing logic and furthermore, specific applications for each class may be engineered, such as image processing applications for morphological processors and biosensors in the case of heterotic devices. The results presented are supported by a range of computer modelling experiments using cellular automata and multi-agent modelling. We conclude that *P. polycephalum* is a polymorphic UC substrate insofar as it can process multimodal sensory input and polyfunctional in its demonstrable ability to undertake a variety of computing problems. Furthermore, our results are highly applicable to the study of other living UC substrates and will inform future work in UC, biosensing, and biomedicine.

Acknowledgements

First and foremost, I would like to thank Prof. Andrew Adamatzky for giving me the greatest gift any man can give another — his trust, in my knowledge, motivation and productive ability. I am forever indebted to him for his tireless help and limitless vision. Furthermore, Drs. Ben De Lacy Costello and Jeff Jones are worthy of similar praise for their calm guidance and supervision.

I would also like to extend the warmest thanks to Dr. David Patton and Mr. Paul Kendrick for their patient training, technical expertise and guidance throughout the course of this program.

I'd also like to thank my colleagues Drs. James Whiting and Ella Gale for their jolly comradeship and various insights.

I must also thank the co-authors of my published articles who have not been previously mentioned, purely for the pleasure of collaborating with them during the course of this program of research: Michail-Antisthenis Tsompanas, Georgios Sirakoulis, Genaro Martinez, Alexis Walter, Nina Gizzie and Tareq Assaf.

Finally, I'd like to extend the warmest, most heartfelt of thanks to my mother Lynda, and my friends Veronica MacDiarmid and Angela Munro, without whom none of this would have been possible.

I suppose my cat, Dagny, also deserves a small mention for raising my morale, despite her attempts to destroy everything in the house in efforts to draw my attention away from thesis writing.

Contents

Chapter 1

Introduction

1.1 Opening Statements

Is a living organism — or a component of an organism — a computing device? Such questions are divisive and are an unhelpful way of comparing the seemingly incalculable complexity of life to an easily quantifiable man-made system. But, it is nevertheless clear that certain living systems are able to solve a wide array of problems in a manner that can be characterised as *computation*.

Through this use of the term 'computation', we here do not make explicit reference to the process by which numerical solutions to arithmetical problems are produced, or indeed simply the use of conventional electronic computers, but rather a generalised notion of the transformation of data in a programmable, predictable manner. 'Data' in this sense is any transient state of a system which can be unambiguously interpreted, such as the distribution of charge across a silicon chip, concentration profiles of various compounds in a chemical reactor or even the space-time configuration of balls on a billiards table. This open-mindedness towards the computational process is the basis of 'unconventional computing' (UC), the field of study which seeks to creatively interpret aspects of the universe as computation or media suitable for the implementation of computing.

When we apply a 'UC filter' to our appreciation of biology, consider that the components of the human brain, neurons (nerve cells) and, to some extent, their supporting glial cells, are able to cooperatively calculate the solution to various problem classes as the product of a remarkably complex series of electrochemical reactions: this process is far-removed from the interactions between charges held in the silicon components of a pocket calculator, yet both are capable of 'computing' the correct solution to arithmetical problems. Thus, there is a common functionality between biological entity and machine.

We are currently ill-equipped to address the nature of the computing processes within media such as the brain as our understanding of the neurobiological basis of thought, problem solving, consciousness etc. have not yet been elucidated with certainty [298]. As Penrose noted in his discourse on the crossovers between brain function, computing and physics [231], gaps in our knowledge of neurobiology and the physical laws which underlie it pose fundamental obstacles towards both full understanding of the mind (the combined functions of the brain; the phenomenological study of which is achieved via the field of psychology) and the creation of artificial minds through endeavours in the field of artificial intelligence. As such, although research which seeks to address these questions is of great importance to so many fields of human inquiry, all such endeavours will be mired in unhelpful mixes of fact and opinion, lengthy debate, controversy, maybe even derision and certainly extensive conflicts of philosophical points of view — indeed, the reader is likely to have an entirely different opinion to the author as well as Penrose on the precise nature of the 'unknown factors' which underlie these concepts (quantum physics, complexity theory, biochemistry, or perhaps even the assertion that there exists no deeper level to reality!) and how best to elucidate them. Coupled with the restrictively broad skill set required of UC researchers due to the field's inherent multidisciplinarity, progress in the production of physical UC prototypes is slow; indeed, the ratio of theoretical to practical publications in the field is an estimated 100 to 1, as of 2015 [25].

The human brain is by no means the only biological substrate capable of computation. The author's original contribution to knowledge contained within this document is one small step towards the monolithic undertaking of linking computer science to biology, but crucially does not constitute a piece on neurobiology, theoretical physics, psychology, artificial intelligence or philosophy. Presented here is an exploration into how the behaviour patterns of a far simpler biological substrate than the brain, a single-celled organism called a slime mould, may be characterised as computation and put to practical use. Such an organism cannot be considered to possess a 'mind' insofar as we understand the term because it does not possess a brain, nervous system or any other apparatus usually associated with concepts of intelligence or indeed computing, yet it is able to exhibit apparently 'intelligent'[1] behaviour. Through research upon such an organism, we seek to avoid the aforementioned pitfalls — complexity of substrate, ethical restrictions etc. — that usually plague research on biological UC substrates and instead focus on the production of laboratory prototypes of slime mould UC devices.

[1]This term is henceforth avoided because of its notorious subjectivity; the matter of finding an objective definition with regards to behaviour classification is discussed in Chapter 2.

1.2 Unconventional Computing: An Introduction

Unconventional — or, non-classical/non-standard — computing is emphatically not the process of fabricating conventional computing architectures utilising novel materials; we do not suggest that a single-celled organism or indeed the brain are suitable substrates for implementing a stored program architecture in a general purpose computer. Indeed, UC is not even necessarily always oriented towards fabricating novel architectures to compete with extant ones. Rather, the primary goals of UC research are, to paraphrase the criteria presented by Stepney *et al.* [285];

1. **To recognise that the physical characteristics of various aspects of the universe autonomously and automatically carry out 'computation' as a by-product of their adherence to the laws of physics.** To pose an example, the beating of the microscopic finger-like projections (cilia) that coat the surface of various species of protozoa passively sort particles according to size as a by-product of the process by which they propel surrounding fluids: larger objects 'float' along the surface directed by synchronised (metachronal) ciliary beating, whereas objects that can fit through the gaps between cilia travel in a different direction [134].

   Applying a 'computationalist' interpretation to an action such as sorting — a problem of situationally variable difficulty on conventional computing architectures — by cilia reveals the stunning efficiency of the mechanism. Specifically, we find that an array of cilia demonstrates decentralised control of a massively parallel array of organelles capable of:

   - Data reception (environmental, inter-ciliary and cellular) and actuation [54, 256].
   - Self-organisation and emergence through the spontaneous generation of metachronal rhythms purely as a function of hydrodynamic forces and tactile stimulation, whose complexity cannot be adequately defined through the interaction of single contributing processes [107, 135, 256].
   - Self-assembly and self-powering through rigorously optimised (via evolution) cytopoietic and foraging mechanisms.
   - Spatial propagation of data which may be represented by the objects being sorted, energy dynamics, concentration profiles in signal transduction media etc. which allows for continuous assessment of the state of the computation.

   These qualities, which exemplify several core UC concepts, are incident of the intrinsic properties of the materials the cell is formed of and the manner of their

assembly (encompassing their linkage and morphology) which, crucially, are attained automatically and at no extra energy cost where such implementations in conventional hardware would be extremely difficult to achieve; for example, clocking mechanisms and sensor sampling are not required in such a decentralised system, self-assembly requires no external guiding influence (e.g. from nanorobots in artificial systems) and motor control of compliant cell components is outsourced to, or embodied within, the materials which form the components (contractile proteins, membrane elasticity etc.) [141, 207].

2. **To exploit natural processes such that we are able to use them for performing ostensibly 'useful' work.** Consider Fredkin and Toffoli's Billiard Ball Model (BBM) of computation [122], wherein hypothetical billiard balls of uniform physical dimensions representing 'data' are propelled along the gridlines of a Cartesian lattice. The output of the 'machine' is interpreted by the presence or absence of data in specific locations after the machine has run for a certain period of time, hence computation may be performed through conditional routing of the balls via their interactions, i.e. collisions followed by elastic ricochets. Thus, such a system may be used to realise logical operations through exploitation of the physical properties of its constituent materials (the BBM is re-visited in more detail in Chapter 5).

3. **To take inspiration from natural systems in order to inform the design of computing paradigms in other media.** This encompasses the augmentation of conventional architectures — for example, bio-inspired computing paradigms such as automated selection-based algorithm optimisation as per the precepts of Darwin's theory of evolution by natural selection (evolutionary computing) [62, 113] — as well as the development of hybrid conventional-unconventional systems, such as human-computer interfaces which, when adequately developed, will be utilised in the generation of neurally-integrated prostheses and other therapeutic, biomedical and biosensing applications [295].

Examples of UC substrates under investigation within recent years include:

- Biological: DNA (deoxyribonucleic acid) computers [37, 188], whole-organism (e.g. soldier crab) ballistic logical gates, [136], genetically-programmed bacterial swarm computers [56], breather (mobile waves of molecule displacement) interactions in biological polymers [119], immunocomputing [292], neuronal computing [64].
- Bio-inspired: Neural networks [42, 143], evolutionary computing [62, 113].

- Chemical: Reaction-diffusion chemical processors based on computation via interaction between growing patterns, such as the Belousov-Zhabotinsky (BZ) reaction [10, 81, 347] and lipid droplet-encapsulated mixed excitable media [63], soap film computing [105].
- Physical: Photonic/optical soliton-based interactions [58], single-electron interactions [142].
- Quantum: Including quantum tunneling [112], ion trapping [283], nuclear magnetic resonance [57] and quantum dot/spin state transition [151] based architectures.

It should be noted that UC does not exclude the use of conventional computers; most subfields make extensive use of computer simulations — cellular automata (CA) (see Appendix B.1 for a description), multi-agent models etc. — in order to specify and record data pertaining to a substrate under investigation in a fully-defined, controlled environment, whilst other subfields such as evolutionary computing seek to implement unconventional paradigms through software run on conventional hardware. Furthermore, UC devices may incorporate conventional computers (as control elements, output interpretation etc.): such a hybrid is referred to as a heterotic computer, although this term can be used to apply to any device comprising more than one variety of physical computing medium interacting synergistically [174].

It must be noted that there are currently a number of active application-based research fields which do not identify themselves as UC, but whose scope and remit encompass aspects of it; examples include the rapidly-expanding topic of molecular communications and the various forms of biomedical engineering/medical physics [71]. This is discussed further in Chapter 2.

As we have seen, the advantageous properties of a particular UC medium are dictated by the physical properties of the constituent materials, be they parallelism, self-organisation, energy efficiency etc., but little has been said pertaining to the applications of such technologies. Unsurprisingly, a substrate's physical properties dictate its application: to list two extremely limited examples, massively parallel processing substrates, such as the BZ reaction or *in vitro* DNA-based processors, are suited to multi-input stream applications such as sensing, whereas quantum computers show great promise for being capable of efficiently implementing algorithms that may only be solved inefficiently (if at all) by conventional architectures, such as factorisation [60, 315].

The rationale behind research into specifically biological UC substrates is explored in Chapter 2. By way of a brief conclusion to this section which has introduced the key

concepts of and justifications for UC research, the following quotation from Margolus [197] summarises the running theme of this investigation.[2]

> "The laws of nature are the ultimate computing resource — the most efficient computation imaginable would make the most direct possible use of the physical interactions and degrees of freedom available."

1.3 Thesis and Research Questions

In acknowledgement of the proposed computational nature of biological processes and the justifications for research on UC substrates, the thesis presented in this document is as follows:

> **Slime mould *Physarum polycephalum* is a polymorphic, polyfunctional unconventional computing substrate.**

To deconstruct this phrase, this thesis can only be substantiated through the construction of physical prototypes of slime mould UC devices that demonstrate:

1. **Polymorphism**: Borrowing the concept from conventional programming languages[3], polymorphism applied to slime mould implies the use of multiple forms of input data — different formats of environmental stimuli — to which the organism responds by instantiating a range of common, well-formalised processes which lead to a repeatable output. The analogy is imperfect as, primarily, it is unclear at this stage in the investigation how data are represented and processed within the organism (or indeed, whether it is the data that processes the components of the organism), but the key concept is that this property implies that irregular, unstructured input must lead to a coherent, statistically-repeatable output via a range of cellular processes which are essentially algorithmic.

2. **Polyfunctionality**: Again drawing from classical computing concepts, this criterion requires that it be practically demonstrated that slime mould UC devices

[2] Although the reader should keep in mind that specifically biological UC substrates are unique in that they have evolved in such a way that the chaos of a vast number of degrees of freedom are reduced and ordered automatically.

[3] In object-oriented programming languages, polymorphism refers to different classes of object which can be programmed in the same manner, using the same operations, from the same interface. Consider that in Java, both INT and FLOAT are polymorphic object classes as they may both be manipulated with the same operations (addition, multiplication etc.).

are capable of carrying out a range of functions, e.g. Boolean logical or arithmetical operations, on input data such that they can be considered to have multiple demonstrable practical uses. This encompasses the need to investigate to what extent slime mould as a UC substrate can be said to be polyfunctional in comparison to, for example, conventional computers.

In plainer terms, the purpose of evaluating this thesis is to construct physical prototype devices using slime mould which are able to solve various classes of computing problem so as to demonstrate to what extent the organism can be used for computing as well as uncover design principles for biological UC in general.

This was evaluated through laboratory-based experimental studies into the mapping of biological phenomena exhibited by slime mould *P. polycephalum* in response to various forms of stimuli as either intracellular physiological (measured microscopically) or/and morphological (macroscopic) parameters; these were consequently utilised as the basis of input and output in a range of prototype computing devices fabricated from slime mould, or utilising slime mould as their key constituent element. In doing so, the following research questions were addressed:

1. Which slime mould morphological and physiological parameters may be utilised as measurable, statistically-repeatable output in a slime mould computing device?
2. How are data and computing tasks represented in a slime mould computing device, how can it be programmed and how is an output perceived and interpreted by the user?
3. To what extent can slime mould computing be used practically and how can the knowledge acquired be applied to the wider field of UC?

These are deconstructed in section 2.4.

1.4 Thesis Structure

This document is structured as follows:

Chapter 2 begins with an introduction to the use of biological substrates in UC, followed by a thorough review of the use of slime mould as a UC substrate and comprises the biological preliminaries describing the cytological, physiological and biochemical aspects underlying slime mould behaviour patterns and a summary of all work completed to date in the field of 'slime mould computing'. Finally, the project's methodology is formalised.

This is followed by Chapters 3–5 which comprise the experimental aspects of this program of research and loosely correspond to the order of the research questions in section 1.3, although each has significant crossover. They examine:

3. **Fabrication of functional slime mould computing devices hybridised with conventional hardware.** Initially, computationally-universal optically-coupled slime mould electrical logic gates are presented, followed by a description of the development of a functional slime mould-conventional hardware interface based on an analogue to digital converter (ADC) and a field-programmable gate array (FPGA). A functional slime mould tactile sensor developed on the platform of this interface is presented.

4. **Morphological processing by slime mould.** Morphological adaptation of the whole organism in response to environmental stimuli is investigated through the use of repellent (optical) and attractant (chemical) stimuli as inputs. It is demonstrated that the organism is capable of interpreting these environmental gradients as spatial 'images' and can consequently perform morphological operations on the shapes represented, including computation of the convex and concave hulls, skeletonization and approximation of shape. Following, it is demonstrated that the organism can be programmed to 'remember' the period of an insulting stimulus and exhibit this as output through modulation of its endogenous systems for achieving motility in a manner consistent with integration in an analogue computer.

5. **Slime mould intracellular computing through the interpretation of endogenous physiological phenomena as output.** An investigation into the processes underlying the behaviour patterns we interpret as computation is presented in which the role of the organism's cytoskeleton in facilitating emergent life processes is expounded. This is substantiated by extensive CA modelling which suggests that this intracellular network of proteins is a suitable medium for information processing. Proceeding is the first practical *in vivo* realisation of a BBM-like computing paradigm which employs intracellular calcium-filled vesicles as data, which travel along and collide whilst attached to cytoskeletal networks.

The document closes with Chapter 6, which contains a summary of the contribution of this research, its limitations and scope for further work.

1.5 Content Disclaimers

Aspects of this document draw on published works by the author. All such material is fully referenced and reproduced data is included with express permissions of the

copyright holders (which is explicitly noted in the text where data are replicated). Any material reproduced from such a publication that was not produced exclusively by the author (i.e. co-authored work) is expressly labelled as such in Appendix A.

Chapter 2

Biological Computation and Slime Mould Computing: A Review

The structure of the following Chapter is as follows: initially, the benefits, detriments and history of using biological substrates for UC are discussed, after which the relevant biology of slime moulds is touched upon in order to provide a suitable grounding to describe later experimental aspects of this document. Following this is a thorough review of the novel field of slime mould computing.

2.1 On the Viability of Using Biological Matter for Unconventional Computing

As soon as biological and artificial computing concepts are compared, a stock of factually-dubious statements usually ensue. For example, Fischetti, writing in Scientific American [116], reported in 2011 that the the Fujitsu K supercomputer is more powerful (faster processing speed and greater informational capacity) than the human brain, but less energy efficient per FLOPS[1]. Such statements are clearly invalid (the author does not mention how thought was quantified in terms of an entirely unrelated metric), but they do serve to demonstrate that the concept of using biological substrates as UC devices has permeated the public consciousness to some degree[2].

The following review of the characteristics of biological substrates pertinent to their use for UC purposes will ideally remove any preconceptions the reader may have following exposure to jargon such as the above example. Although UC is an active research area, there have been so few functional biological UC devices developed to date that we cannot actually say much about their practical characteristics with regards to what has been explicitly demonstrated. Indeed, there is still much that we do not know of biology in general, as is discussed in section 2.1.5. Hence, the following information is presented to demonstrate principles of data processing in biological systems with the intent of informing the design of practical implementations of said systems.

2.1.1 Biological Information

Broadly speaking, the term biological 'information' pertains to the state, quantity and/or localisation of biological macromolecules in a contained system, such as DNA/RNA (ribonucleic acid), allosteric proteins (e.g. protein kinases, enzymes) or cytosketetal proteins[3], although this is by no means an exhaustive description as any transient state of a biological system may be interpreted as data.

Input data into a biological system (live or *in vitro*) is not necessarily macromolecular, but will typically be transduced (perceived, converted into another form and consequently transmitted) into some variety of energetic phenomenon by the system's receptive components, which in turn precipitates a change in the state of the system. The

[1] "Floating-point operations per second", a quantitative measurement unit for processing power.

[2] Possibly via the route of science fiction on the topic, which began to emerge shortly after the dawn of cybernetics in the 1950's [74].

[3] The cytoskeleton is an intracellular 'scaffold' of proteins that provide a cell with structural rigidity and a network for substance transport. This is covered in more detail in Chapter 5.

manner in which transduction occurs is pre-determined by the way in which the system is assembled and coupled, this being a product of the cell's genetic make-up. This concept is complimentary to 'data structuring' in artificial intelligence, which refers to the conversion of abundant but sporadic, multimodal environmental sensory data into a statistically repeatable single format that the system is able to interpret unambiguously. Possession of such a system allows the cell to coordinate appropriate, proportional, reliable responses in accordance with its genetic 'programming' [123, 141].

For example, photostimulation is an attractant in certain halobacteria species [288], a mechanism which presumably evolved due to the class being facultatively photosynthetic: hence, the bacterium 'computes' its ideal location based on the degree of environmental illumination. At the molecular level, this behaviour is achieved through the interaction of photons with the membrane-bound photoreceptor protein bacteriorhodopsin, which causes it to enter a high energy state that catalyses an intracellular signalling cascade, eventually leading to chemical energy production in the form of adenosine triphosphate (ATP)[4]. This, in turn, precipitates directional movement via innervation of local motility mechanisms [246, 288][5].

In such an example, the optical stimulus may be considered as input which is transduced into the format of biological molecules that may be regarded as a form of 'encoded' data; indeed, the bacterium could be said to have carried out computation in the bacteriorhodopsin molecule in this process of transduction which we characterise as an encoding operation. The instruction "move towards the light source", which is encoded by the structure and function of each molecule in the system that responds to such input (and hence also the genes that determine the assembly of this system), is enacted through the interaction of ATP with various components of the cell's motile machinery[6].

2.1.1.1 Spatial Propagation of Information

The example of biological information processing given in section 2.1.1 is conducive to the UC paradigm of spatial propagation of information, i.e. the net movements of ATP and associated signalling molecules through the cell towards areas where it will lead to the production of a repeatable, measurable output. This is a particularly advantageous concept (and indeed it is not unique to biological UC) for the primary reason that it

[4]The ATP molecule can be considered as the chemical energy 'currency' of life. All metabolic processes in eukaryotes are concerned with converting external sources of energy into this molecule, e.g. photosynthesis and oxidative phosphorylation of sugars. Dephosphorylation of ATP (usually enzymatically) releases this energy for use in cellular processes.

[5]It should be noted here that different isoforms of bacteriorhodopsin are responsible for slightly different functions, which have been generalised here for ease of illustration.

[6]In the case of this somewhat simplified example, the process is both non-linear and non-deterministic.

allows the computing process to be continuously examined. This facilitates, with respect to biological UC:

1. **Continuous updates as to the state of the computation.** Various methods of visualisation (microscopy, flow cytometry, radiometry etc.) may be used to assess the state of the system at any point mid-computation, allowing for assessment of how the computation is performed, its efficiency, whether errors have emerged and the overall system dynamics. This latter point refers to the fact that the process of interest forms only one small aspect of a larger system which, crucially, will likely adapt mid-computation.

2. **More efficient utilisation of a substrate's resources through observation of interrelated processes.** As the processes which will determine a biological system's momentary state are usually highly complex, multi-step pathways which involve a great many initial, intermediate and final products as well as a host of enzymes which catalyse each step[7], this implies huge potential for exploiting the computing potential of other related pathways. It must be emphasised again, however, that this necessitates that the system is understood and characterised to a sufficient degree.

The experimentalist must keep in mind the major detriment to this concept, which is that directly observing the system carries the risk of altering it in some way.

The reader should note that this interpretation is very far removed from the Turing model of computation [304]: the individual processes we choose to interpret as computation are to all intents and purposes not halting and involve the propagation of essentially random movements of parallel data streams in a constantly evolving system. Crucially, however, we nevertheless find that the computation is completed successfully to, what we can assume on an evolutionary level, a high degree of efficiency (although by taking an organism or its component out of its natural habitat such that the system enters a stressed state, we risk compromising this inherent attribute).

2.1.1.2 Information Density

Biological molecules possess the potential for extremely high information density, even in comparison to artificial media. For example, the human genome contains some 1.5 gigabytes ($\times 10^9$) of data [186], meaning that a human composed of 100 trillion ($\times 10^{12}$)

[7] Consider that glycolysis, the pathway by which glucose is converted to pyruvate, which forms only a small portion of the mammalian energy production pathway (oxidative phosphorylation) involves 8 transformations of the original glucose molecule and involves 5 related compounds and 10 different enzymes [91].

cells contains approximately 150 zettabytes ($\times 10^{21}$) of (admittedly repeated) genetic information. It is not appropriate to compare this value directly to that of a personal computer's hard or solid state drive as estimations of information density introduce significant error[8], and indeed this estimate does not account for the redundancy within codons[9] or between chromosomes, but initial projections indicate that engineered DNA computing may achieve information densities exceeding 700 terabytes ($\times 10^{12}$) per gram [69]. Retrieval of this information (genetic transcription) is slow but is highly energy efficient as it does not present the surface-to-volume heat dissipation problems exhibited by conventional hardware (see section 2.1.2).

2.1.2 Architecture

Conventional computing architectures were historically considered to carry out serial computation, i.e. each task is broken down into a set of instructions which are carried out sequentially; when given multiple tasks, a serial architecture will split its time evenly between them by placing a process on hold and storing its state before moving to the next, and so on. A major focus of computer development has been upon increasing the speed at which it is able to carry out sequential instructions, such as maximising the efficiency of algorithms and increasing the clock speed of a computer's central processing unit (CPU). The universe is of course an inherently parallel place, however, as every process occurring in any one instant happens alongside every other process [30, 285]. Whilst there has been a trend in recent years towards implementing parallelism in both personal computers (PCs) — for which, at the time of writing, high-end CPU chips may have up to 8 cores and graphics processing units may possess several thousand, each of which functions as a discrete processing element such that it can carry out many processes simultaneously in properly-optimised workloads — and supercomputers, which may possess thousands of separate CPUs [80], this pales in comparison to the inherent parallelism of natural systems. For example, if each neuron in a human brain is considered as a discrete processing unit, it can be said that the brain possesses 100

[8] All of a cell's genetic data (one of many forms of biological 'information') is held in its nucleus, an approximately 10 μm^3 structure, which typically accounts for 10% of its volume but will only contain 0.5% DNA per unit mass [49].

[9] A codon here refers to a triplet of messenger ribonucleic acid (mRNA; the nucleic acid format transcribed from a cell's genome which then travels out of the nucleus to participate in protein synthesis (translation)) bases, to which complementary codons of transfer-RNA carrying amino acids bind within cytoplasmic organelles called ribosomes, therein precipitating the formation of a protein chain. This process generates some redundancy in the system as well as error resistance, as mis-transcribed genetic sequences may terminate if certain 'nonsense' codons enter the ribosome as no complementary tRNA codons will bind to it; this prevents the formation of incorrectly-assembled proteins.

billion processor cores, between which there are some 100 trillion connections (synapses) [97][10].

This concept is not unique to biology as indeed many chemical and physical systems also exemplify the concept: in chemical reactors it can be said that each molecule of reactant represents a discrete processing element (a concept of the UC subfield of reaction-diffusion computing [30]), hence the number of processors per unit volume is equal to Avogadro's number; in the order of 6×10^{23} per Mole of solute.

An architecture possessing more than a few processing cores is commonly referred to as being massively parallel [30, 79], but this can be a somewhat misleading term as it is commonly applied to both processing methods which can be implemented on single discrete PC graphics cards [109] as well as the comparatively *exorbitantly* parallel capabilities of natural systems. The term 'amorphous computing' is perhaps more apt as it is used to imply massive parallelism in a system with no fixed architecture, but as efforts are already underway to implement amorphous man-made systems it perhaps equally as unhelpful [4, 5, 79]. Further mentions of massive parallelism are made with reference to systems which are significantly more parallel than current GPU/supercomputer capabilities, i.e. capable of processing in excess of several thousand data streams simultaneously.

It must be emphasised that neither massively parallel nor amorphous architectures are necessarily well-suited to every computing application — indeed, the 4-core CPU belonging to the PC on which this document was composed is able to execute about 24.5×10^9 instructions per second [153], and is likely to be a far more efficient tool for writing a thesis on than a thousand-core supercomputer — as indeed, novel and emerging UC devices need not necessarily be aimed at succeeding conventional architectures in every aspect. There are major benefits to amorphous computing, however, including:

1. Enabling a higher number of instructions to be computed per unit time in comparison to serial computation [4].

2. Better adaptation to 'real world' applications, such as sensing of multiple parallel input streams [285] and modelling of natural systems composed of extremely large numbers of individual agents (which has been achieved on a small scale with logical parallel arrays implemented on graphics processors [109, 187, 299]).

[10] Again this is a simplistic comparison as conventional and unconventional computing concepts are not directly comparable, and furthermore it is debatable whether a cell can be considered as a processor: the key concept imparted here is the magnitude of the parallelism in a biological substance.

3. Fault tolerance and reliability, as the failure of multiple processors does not dramatically impair the functionality of the system [75, 285][11].

4. Energy efficiency, in both resource and running costs (see section 2.1.3).

2.1.3 Energy Efficiency

Having undergone some 4 billion years of evolution, biological substrates capitalise upon highly efficient processes in order to harness environmental energy into the ATP molecule. To discuss the energy efficiency of this and related processes with regards to their use for computation is another topic over which extreme care must be exercised: the power consumption of the human brain, for example, has been quoted as circa 12–20 W [73, 243], a superficially low value in comparison to that of a desktop computer such as the one this document was produced on, which requires a 650 W supply (not including the monitor). Conversely, IBM's Watson supercomputer is a 750,000 W (not including cooling apparatus) leviathan designed to interpret aspects of natural language and can structure input equivalent to a million books of text per second, yet it still lacks basic comprehension of discourse that is not structured as a question [80]. Again, this highlights the discrepancies between the manner in which different computing substrates function and hence the unhelpfulness of such simplistic comparisons.

Thankfully, more scientific measurements have been elucidated. In their review of power consumption in real and artificial neural computing systems, Sengupta and Stemmler [265] comprehensively examined the energy efficiency of neural communication and concluded that the brain is supremely energy-efficient (as would be expected, from an evolutionary perspective) through examining its ability to:

1. Utilise both digital and analogue signals (the energy efficiency of each differs depending on the signal-to-noise ratio of the neural relay).

2. Minimise energy dissipation as heat through balanced signal amplification and reuse of the products of spent chemical energy.

3. Optimise the scale and content of cells.

4. Develop variable excitability coefficients between neurons of different sizes, shapes and their frequency of use (synaptic plasticity).

These characteristics were compared to the energy efficiency per bit of information (represented as ion currents normalised against metabolic efficiency in the biological

[11] Although, in Ref. [75], the authors warn that, on thermodynamical grounds, parallelism is only a desirable characteristic insofar as the system's architecture facilitates full use of its resources.

equivalent, i.e. bits per molecule of ATP expended) processed in conventional hardware and found that the brain is indeed more energy efficient than conventional and super computers by many orders of magnitude.

The importance of reducing energy dissipation in novel architectures — be they artificial or organic — should not be understated as the thermalisation of waste energy in conventional computers poses significant environmental and engineering problems. In their treatise on a new, unconventional logic — conservative logic [122], which exploits input-output bijectivity (one-to-one mapping) and time-invertibility (logical reversibility) — Fredkin and Toffoli predicted in 1982 that the progressive miniaturisation and consequent denser packaging of circuit components in silicon chips would lead to the generation of heat sufficient to damage them long before the physical size limitations of silicon components were reached. They named this impediment the 'kT barrier', referring to the thermodynamical law that for every bit of information erased by a system[12], a certain amount of energy would be thermalised and dissipated. This is indeed a significant problem nowadays which has only been curtailed through ingenious engineering; PC CPU chips began to be supplied with heatsinks in 1993 [152] and it is not uncommon for powerful modern PCs to have water cooling systems installed. Whilst the issue of heat-induced damage may yet be addressed by engineers[13] , the search for cooler computing methods is a feasible route towards making architectures that are better suited for miniaturisation. A reduction in waste energy dissipation is concomitant with increased energy efficiency, which is justification enough for favouring this approach until we are able to mass-produce entirely carbon-neutral, renewable power.

2.1.4 Self-assembly and Emergence

There is currently a growing consensus in the nanotechnology community that self-assembling circuitry is a viable route towards generating artificial hardware, as a body of experimental evidence indicates that such methods may produce architectures with smaller node sizes than can currently be achieved with conventional methods (i.e. photolithography) [55, 255, 341]; it should be noted, however, that this technology is very much in its infancy and fully-guided assembly has not yet been achieved.

[12]This prediction was given with reference to the inherent inefficiency of conventional logic: for example, in an AND gate, the input configuration $\langle AB \rangle$ leads to a single signal being output. This implies that one signal, therefore, has been destroyed, the majority of whose energy is dissipated as heat. Conservative logic was presented as a new computing paradigm as it enables circuits to be designed with no signal loss, hence the namesake.

[13]E.g. through the use of super-strong nanomaterials such as carbon nanotubes, which are at the time of writing being touted by the scientific media as the most likely material for producing next-generation nanoscale transistors and hence provide new impetus to recent plateauing in Moore's observation [216, 228, 272].

The self-assembly of biological matter on a sub-nanometre scale (i.e. protein synthesis via genetic transcription and translation) is a well-characterised phenomenon. As such, biological materials could be regarded as being well-suited to generating extremely small circuitry (for want of a better term to describe paths of information flow) — although, this does pose the necessity of exerting some level of control over the substrate if one is to harness said processes for any practical benefit.

Self-assembly is one of the many emergent properties that biological substrates exhibit (see Appendix B.4 for a definition of emergence). Whilst the phenomenon of emergence is not examined further in this section as each system will possess its own unique emergent properties, this concept is re-visited in section 2.2; suffice it to say here that we regard emergence as a beneficial characteristic for any hypothetical biological UC substrate as it adds the potential for generating extra layers of complexity and hence more computing resources at no additional 'cost' (in energy), due to it arising from a fundamental characteristic of constituent materials.

2.1.5 Detriments

Aside from this positive appraisal of the beneficial characteristics of biological matter as a UC substrate, it is in reality not without its detriments. It was briefly mentioned in Chapter 1 that we still have an incomplete understanding of many aspects of biology and indeed the underlying mechanisms that drive it[14], but this is far from being the only impediment towards fabricating functional, useful biological UC devices.

Biological substrates are unpredictable; both live organisms and their components have highly specific physiological requirements, which makes working with them time consuming, costly and requires the researcher to possess specialist knowledge and practical skills. For example, mammalian cell cultures must be incubated in a precisely pH-balanced buffer with the correct nutrient content, at a specific temperature in an entirely sterile environment; and even then they are temperamental and may attenuate, mutate or just spontaneously perish at the slightest deviation in these parameters [108]. Coupled with the high costs of running cell culture experiments and the ethical issues surrounding the use of mammalian cells [127] (which are less stringent than those required for working with live organisms or human subjects), it is perhaps reasonable to

[14]Note that whilst an impediment, we do not consider our lack of understanding to be a 'detriment' of biological UC substrates in the context of finding practical uses for them as indeed, such research may be considered as a viable route towards investigating their biological properties, as well as computing characteristics; this is discussed further in section 2.1.6.

assume that computer scientists with non-biological backgrounds may be deterred from working with the majority of biological substrates[15].

The speed at which biological processes occur is fairly variable but tends to be slow in comparison to the speed at which conventional architectures will function. For example, the propagation delay[16] of a myelinated (central) nerve cell's action potential is typically about 5 ms [240] whereas waiting for a gene to be expressed — e.g. in a transgenic cell line expressing proteins in a manner analogous to a logical gate [290] — typically takes several hours and may not even be expressed in the current generation of cells. Conversely, the propagation delay in a modern CPU is in the order of picoseconds [338]. Although some as-of-yet unelucidated biological phenomena may occur much more rapidly than the examples listed (e.g. propagation of quantum events down protein chains such as breathers (coherent waves of vibrational energy induced by thermodynamic disturbances) in DNA molecules [9, 119]), no known biological phenomena propagate at a speed approaching that of electricity-based serial communication in conventional architectures. This further emphasises the need to exploit the massively parallel capabilities of biological UC substrates.

Perhaps the most significant obstacle standing in the way of biological UC research, however, is the inherent variability of the substrate class. Conventional computers have been rigorously developed to the point that they are nowadays extremely proficient at carrying out their role, i.e. executing algorithmic procedures; this necessitates their being regularly structured, highly repeatable systems that will carry out a calculation exactly as programmed to give the same result each time the calculation is performed. Whilst some biological variables are tightly controlled by closed-loop systems (e.g. cytoplasm pH [320]), variation is a fundamental characteristic of biological matter. This variation results from myriad inconsistencies in an organism's nature and nurture such as genetic transcription errors and transient metabolic disturbances. But, far from being detrimental to the organisms health, their inherent variability in fact forms the basis of their ability to adapt to extreme environmental variation, enhances the reliability of life processes — i.e. fault tolerance through multi-system redundancy (consider that mammals have 2 eyes, 2 lungs etc.) — and forms the basis of evolution by natural selection of desirable characteristics whose incidence is essentially random [77].

Consider, for example, that several Gram-negative[17] bacteria such as *Pseudomonas*

[15]Conversely, it has been demonstrated that a significant proportion of biologists are averse to heavily mathematical subjects such as computer science [111].

[16]This computer science term is used here with respect to biological systems to refer to the time a unit of information will take to travel through a functional unit, regardless of whether computation can be said to have occurred or not.

[17]This terminology refers to a classification for bacteria based on the presence of a cell wall by differentially staining heat-killed organisms for peptidoglycan, an obligate component of the bacterial celll wall.

aeruginosa may vary greatly in size, growth rate, cellular content (macromolecular and organelle) and even behaviour in genetically identical colonies due to a highly error-prone cell division process [68]. A hypothetical bacterial UC device which focussed upon, for example, bacterial migratory patterns (upon which several bio-inspired algorithms have been proposed [215]) would therefore have to be based on statistical measures or otherwise conventional paradigms with very wide error tolerances, as the possibility of a significant number of dysfunctional bacteria not following predicted patterns — e.g. due to different movement rates, failures of chemotactic mechanisms — cannot be ruled out. This problem is compounded by the aforementioned temperamental nature of biological organisms as behavioural adaptation resulting from variation in environmental conditions is unavoidable due to the literal impossibility of keeping all such variables constant in an experiment. Such a computing system would be far-cry from the well-regulated environment of a conventional computer; this highlights that biological UC substrates should either capitalise upon the few highly conserved, tightly regulated, robust processes (e.g. DNA transcription) or utilise paradigms that do not require (or perhaps even exploit) chaos in a system [285]. Indeed, as was explored in a recent review by Privman [239], some research groups have already begun to suggest specific methods for curtailing sources of noise and error in biological and biochemical systems towards creating scalable, stable information processing networks.

Note here the apparent contradiction between the previous point and the purported benefits of biological UC substrates, i.e. attempting to constrain the multiple degrees of freedom in a biological system towards making a computer more akin to a conventional architecture, surely negates the benefits associated with the material. We emphasise here that although it would be ideal to use every available degree of freedom in a substrate as a computing resource (cf. Margolus quote in Chapter 1), practically speaking the UC researcher must strive towards finding an ideal balance between utilising and constraining a substrate's degrees of freedom, variability and hence controllability, all of which are unique to the substrate of choice.

2.1.6 Applicability to Wider Fields of Inquiry

In Ref. [88], Dawkins imparts the concept that our modified Linnean taxonomy system creates the misconception that species groups are rigidly-defined, set-in-stone categories: the distinction between species groups is in fact a continuum describing comparative differences between gene pools which, crucially, are constantly changing. We borrow this analogy to impress that similarly, the natural sciences are not rigidly delineated as there is significant cross-over between each field of inquiry. Consider that the task of developing a drug requires input from biologists, chemists and those who work in the

middle-ground; pharmacologists, clinicians etc. We advance the notion that the same can be said for unconventional computing[18], as we conceive that advances in the field will lead to advances in other fields of scientific inquiry.

Indeed, it is apparent that several extant biological fields already draw on computer science: such developments are manifold and diverse, but include the implementation of DNA storage of user-encoded information [69], solving NP-complete problems with DNA[19] [37, 188, 190], *in vitro* synthesis of various nucleic acids, enzymes and other proteins [275] and implementation of *in vitro* and *in vivo* logical circuits using enzymes [52, 149]. The eventual applications of these technologies range from biomedicine (e.g. detection and reversal of malignant transformations in tumour cells via genetic engineering [321]) to modelling (gene regulatory networks [154], collective behaviour patterns [109]) to sensing [230, 313].

Although these achievements may all be reasonably called advances in unconventional computation, their authors refer to their fields as synthetic biology, systems biology, biological nanocommunication networks or developmental biology, the definitions of which are notoriously fluid [229]. It is clear that these 'species' of inquiry constitute a continuum drawing from many fields. We propose, therefore, that advances in unconventional computation may be highly applicable to any or all of these interrelated fields and hence advances in biological computing may lead to developments in biosensing, biomedicine, etc. and *vice versa*: thus, this constitutes a supplementary justification for UC research.

2.2 Biological Preliminaries for Slime Mould Computing

2.2.1 Why Slime Mould Rather than Another Organism?

We are now aware of the justifications for biological UC research, but how is one to choose an appropriate substrate? As was alluded to in Chapter 1, it is sensible from the perspective of the experimentalist to choose an organism/components of an organism that are as simple as possible in order to avoid the pitfalls of overwhelming substrate complexity and philosophical arguments resulting from careless use of anthropomorphising terms such as 'consciousness'. This was a simplification, however, as complexity is a notoriously subjective term; hence it is unwise to base such a decision purely on an abstract concept.

[18] Parenthetically, the UC theme of studying computational aspects of the universe could even be considered to be a natural science.

[19] Both the Hamiltonian Path and Travelling Salesman problems; 'NP-complete' here refers to a complexity-sorted category of problems that have a "yes" or "no" answer, the solution to which can only be verified in polynomial time by a conventional Turing machine [184].

Here we present partial justification for selecting our substrate for this investigation, slime mould *Physarum polycephalum*, by demonstrating that it exemplifies certain desiderata for a biological UC substrate; briefly, these include possession of analogous cellular machinery to human cells, simplicity, frugality and safety of cultivation, tolerance to harsh environments and multimodal stimulation, independent lifestyle and exhibition of novel and emergent behaviours characterisable as computation. Simultaneously, this section provides a brief introduction to the biology of slime moulds pertinent to achieving a necessary grasp of the topic to support the following investigation. In the section 2.3, we complete our justification by demonstrating its proven value specifically as a UC substrate. A glossary of the biological terms used in this section is included in appendix B.5.

2.2.2 Taxonomy, Life Cycle, Habitat and Cultivation

The plasmodial, or 'true'[20] slime moulds which inhabit the class *Myxogastria* (class *Amoebozoa*, kingdom *Protozoa* [284]) are a group of organisms which exist as a macroscopic single cell when in their diploid vegetative life cycle phase, the 'plasmodium' (pl. *plasmodia*) (Fig. 2.1) [115, 226, 284]. Despite the implication from their name, slime moulds are not fungi but rather protists, although they were originally classified as the former due to the organism's reproductive cycle necessitating irreversible transformation of the plasmodium into spore-producing fruiting bodies (sporangia) when environmental conditions become unfavourable [226, 284]. Sporangia-constituent spores germinate into either flagellated 'swarm' cells when in liquid media or crawling myxamoebae[21] on solid media, both of which are haploid but may reproduce sexually or asexually, depending on the favourability of environmental conditions [284]; both germinate into immature plasmodia. The plasmodium has another, reversible cell stage known as the sclerotium, a desiccated, highly resistant form which the organism transforms into when deprived of moisture. Sclerotia remain viable for many years and may be revived in a matter of hours through rehydration, hence this allows the slime mould researcher to build stocks of the organism for long-term storage and transit. Images of the organism's major life cycle forms are shown in Fig. 2.2.

Although present to some degree in every continent (except for Antarctica) in a variety of habitats, the genus *Physarum* is found most frequently in European, North American and Japanese woodland [223]; the plasmodium typically crawls between moist, dark

[20] This is contrasted with the 'cellular' slime moulds of class *Dictyosteliida* which are macroscopically similar, but in fact exist as many loosely-connected individual cells living in unison, and the microscopic slime moulds of class *Protosteliales*.

[21] Both the plasmodium and myxamoeba are frequently referred to as 'amoebae'. Although amoeba-like in some aspects, this is categorically false.

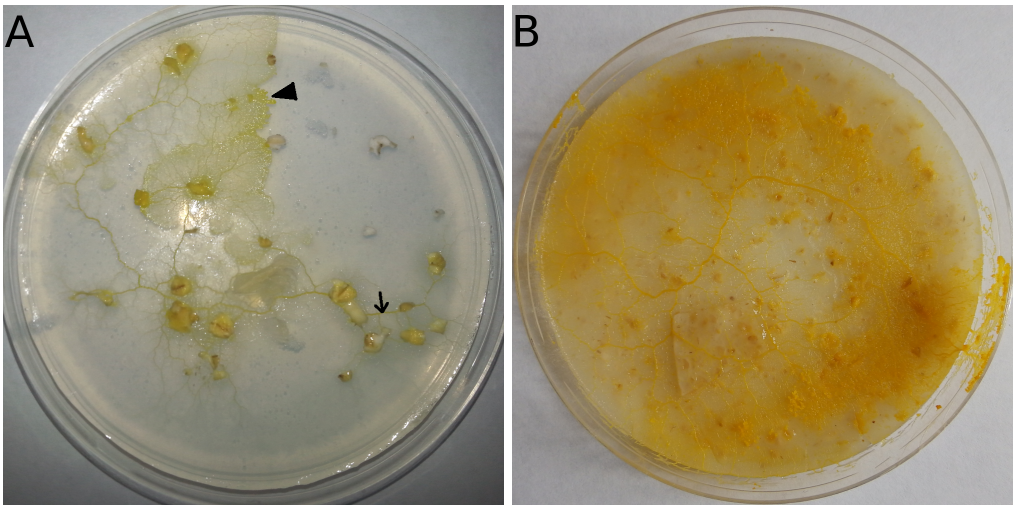


FIGURE 2.1: Photographs of the *P. polycephalum* plasmodium. (a) Cultivated on non-nutrient media in the presence of spatially distributed nutrient sources (oat flakes); the organism's morphology is somewhat diminutive with caudal regions (arrow) and assumes a tubular morphology whilst the advancing anterior margin is noticeably 'fan-like' (arrowhead). (b) On a nutrient-rich substrate (oatmeal agar), the organism becomes essentially amorphous. Figure adapted from author's own work in Ref. [201].

areas of forest detritus or bark, feeding on decaying matter, fungal spores, bacteria and amoebae in a manner not dissimilar to leukocytic phagocytosis, i.e. engulfing and internalising the substrate in phospholipid membrane-bound sacs (vesicles) [104, 284].

In the laboratory, *P. polycephalum* plasmodia are remarkably easy to cultivate. Various sources state a preference for using 2% non-nutrient agarose (NNA) as a medium, but any hydrated surface is sufficient as indeed, even dampened tissue paper has been utilised to great effect in scientific studies [14, 284]. Similarly, fully-defined (axenic) solid and liquid culture media do exist [210], but the plasmodium will grow rapidly and healthily when fed on ordinary rolled porridge oats [284]. We estimate the minimal power consumption of a 20 mm^2 plasmodium weighing about 0.1 g to be approximately 0.16 W (see Appendix B.2 for calculation); this value is extremely low per unit of mass in comparison to other substrates (biological and artificial) we have examined.

The combination of utilising a non-nutrient medium and complex dried foodstuffs radically reduces the issue of microbial contamination, although *P. polycephalum* is also known to feed on certain microbe species and is likely to possess anti-microbial mechanisms [83, 104].

Cultivation temperature need not be controlled tightly as *P. polycephalum* appears to be tolerant to a wide range of 'room temperatures', although it will proliferate more rapidly within an idealised range of 22–24^{o}C [332]. Temperature is a variable which should be controlled to some degree in experimental conditions due to it having an effect on the

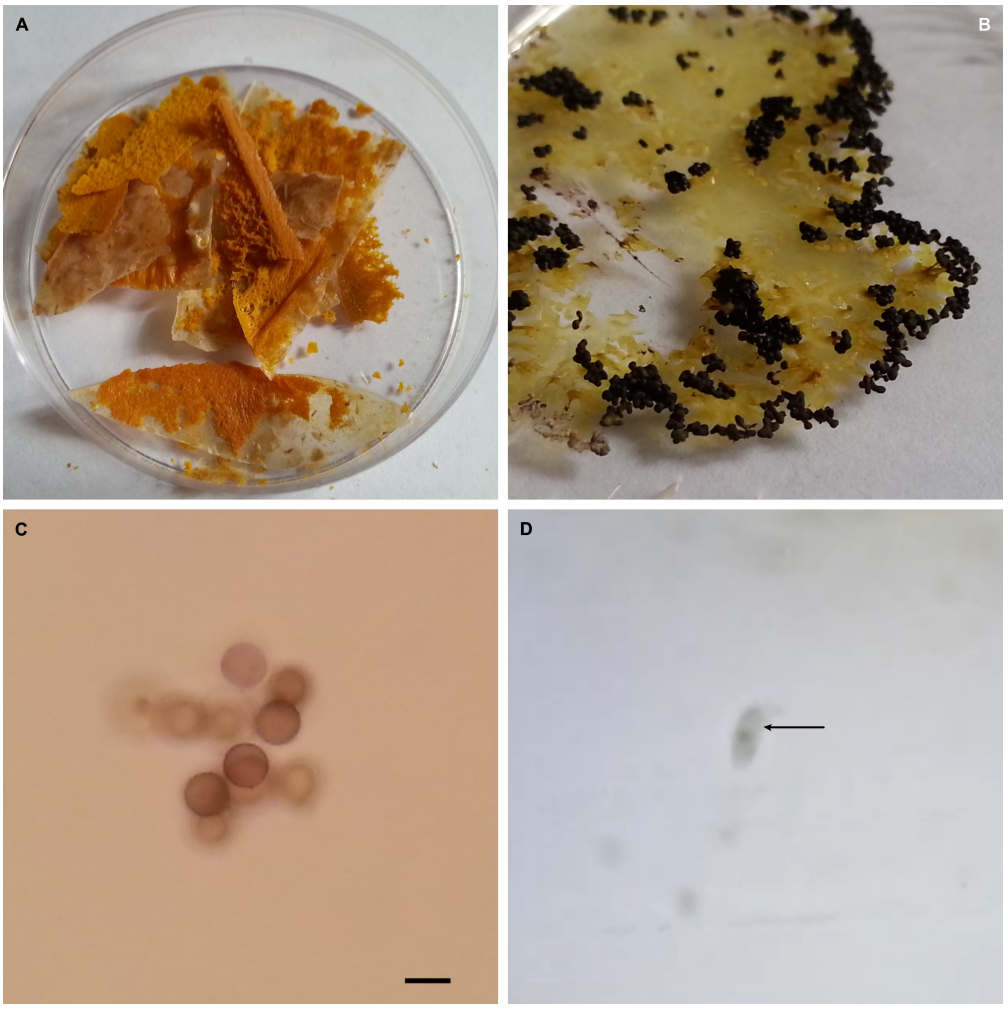


FIGURE 2.2: Images to illustrate the various life cycle forms of *P. polycephalum*. (a-b) Photographs. (c-d) Micrographs, scale bar = 10 μm. (a) Desiccated sclerotium. (b) Sporangia (sporing bodies). (c) Spores. (d) Flagellated 'swarm' cell (arrowed). Figure adapted from author's own work in Ref. [201].

organism's behaviour patterns [334] (see section 2.2.5). Being photophobic, it is best cultivated in the absence of light.

As an entirely harmless (non-pathogenic, non-toxic and non-allergenic) single-celled organism, there are no ethical issues surrounding the experimental use of slime mould [173] and indeed, communities of hobbyists and artists exist who grow the organism at home [296].

In comparison to the requirements for mammalian cell culture presented in 2.1.5 (and also microbiological culture techniques, which are arguably of lesser complexity but carry greater risks to the researcher), it is clear to see that slime mould cultivation is comparatively frugal, safe and simple. These are all prime justifications for the experimental use of slime mould as a biological UC substrate; indeed, it has been noted by several

authors that slime mould is an ideal 'entry-level' biological substrate for use in both multidisciplinary research and education [25, 173].

2.2.3 Morphology and Motility

As shown in Fig. 2.1, the *P. polycephalum* plasmodium's morphology (see appendix B.5 for definitions of the anatomical notation used hereafter) is complex and situational. In the wild, the organism's conformation is similar to that which is shown in Fig. 2.1A; the caudal regions of the organism take the form of tubular structures (henceforth known as 'plasmodial tubes') which link larger engulfed nutrient sources together to form a complex, reticulated network. The topologies of nutrient harvesting networks have been demonstrated to be highly efficient [11, 220], providing an exemplar case of computation by slime mould (see section 2.3).

The advancing anterior margin of the organism, which may move at speeds exceeding 10 mm h^{-1} [43], is often called amorphous or otherwise 'fan-shaped', but is in actuality the confluence of myriad microscopic tube-like structures which are constantly re-forming (Fig. 2.3). Favourable stimulation (see section 2.2.5) of the advancing margin membrane results in local cytoskeleton assembly which causes the extension of 'finger-like' protrusions known as pseudopodia [44, 70, 131]. If an individual pseudopodium continues to encounter favourable stimuli (e.g. a nutient gradient in its substrate), it will gradually fortify until it forms a macroscopic tube in the direction of the stimulus [44].

When the organism is cultivated on a nutrient-rich substrate, it assumes the form of an amorphous mass which expands radially; the ability to possess multiple advancing portions is thought to be the origin of the organism's name 'polycephalum', i.e. many-headed.

Although cytoskeletal assembly (see chapter 5) drives the direction of migration, motile force is generated by the organism rhythmically propelling its constituent fluid (cytoplasm) with muscle-like protein complexes (actomyosin) that sit circumferentially about the perimeter of plasmodial tubes; longditudinal, radial and spirally-oriented fibres of actomyosin contract to exert force on the cytoplasm [225, 248, 330], which can be observed with a light microscope as gradual oscillation in the diameter of the plasmodial tubes (Fig. 2.4) [44]. Net movement is maintained through disassembly of protein networks in the trailing portions of the organism and either complete withdrawal of the plasmodial tube from the area or, if the organism deigns to keep an area occupied (e.g. if it contains partially undigested nutrient sources), the cytoplasm in the region is converted into gel phase (gelation).

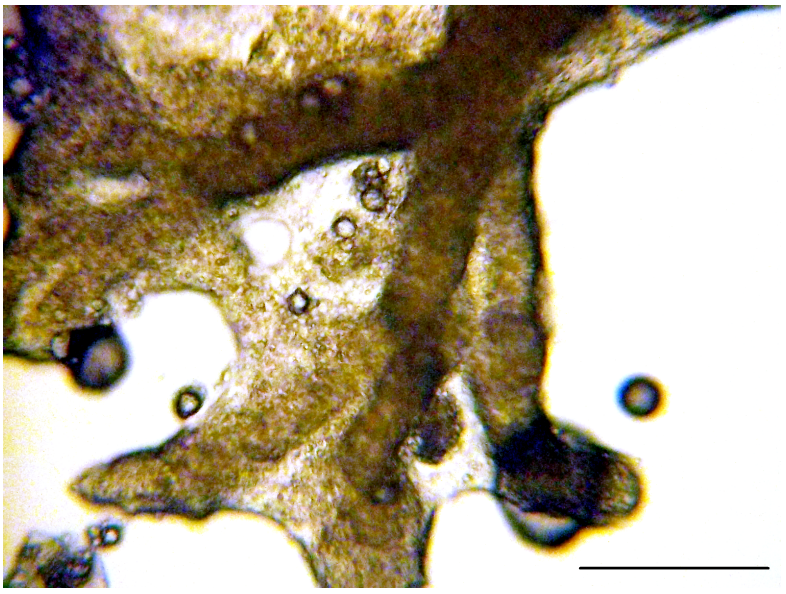


FIGURE 2.3: Photomicrograph of the *P. polycephalum* plasmodium advancing anterior margin. Multiple tubular channels are evident which anastamose to form pseudopodia. Scale bar = 500 μm. Figure adapted from the author's own work in Ref. [203].

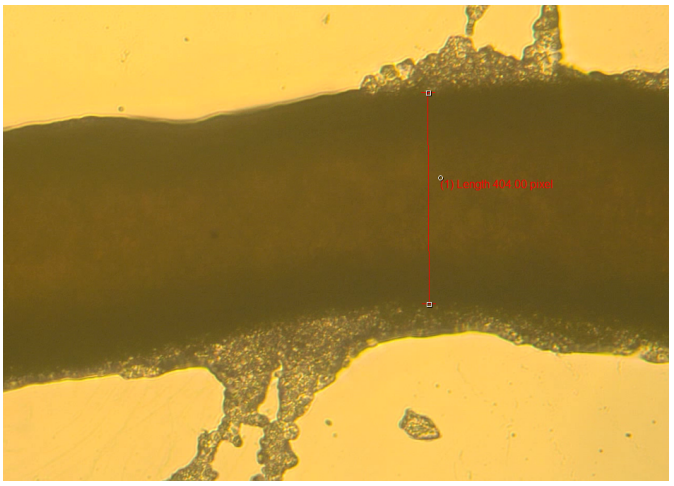
The direction of cytoplasm propulsion oscillates anteroposteriorly, reversing direction approximately every 1–2 minutes. Opinion is split on exactly how net forward migration is achieved by these alternating patterns of contraction and relaxation; substantial historical literature states that tube contraction is simultaneous and monorhythmic (at least in larger tubes), whereas newer evidence indicates it being more akin to peristalsis (i.e. sequentially propagating) [44, 46, 131, 308].

The rhythmic back-and-forth propulsion of cytoplasm is known as 'shuttle streaming' (similar in most aspects to 'cytoplasmic streaming' (cyclosis) in amoebae); aside from the generation of motive force, it also serves to distribute the contents of the cytoplasm throughout the organism. It is from this phenomenon that the organism's genus name 'Physarum' arises, which translates approximately from Greek as 'bellows' [93].

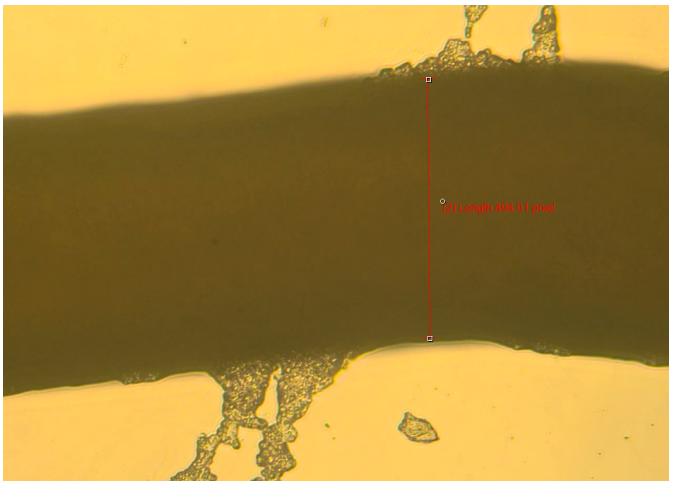
A great many of *P. polycephalum's* intracellular processes oscillate in some degree of phase with shuttle streaming, although shuttle streaming is not the root cause of these related processes: this topic is thoroughly investigated in Chapter 4, but it is necessary to note here that plasmodial bioelectrical potential, when measured non-invasively at the membrane, oscillates approximately in synchrony with shuttle streaming (Fig. 2.4c).

2.2.4 Cytology

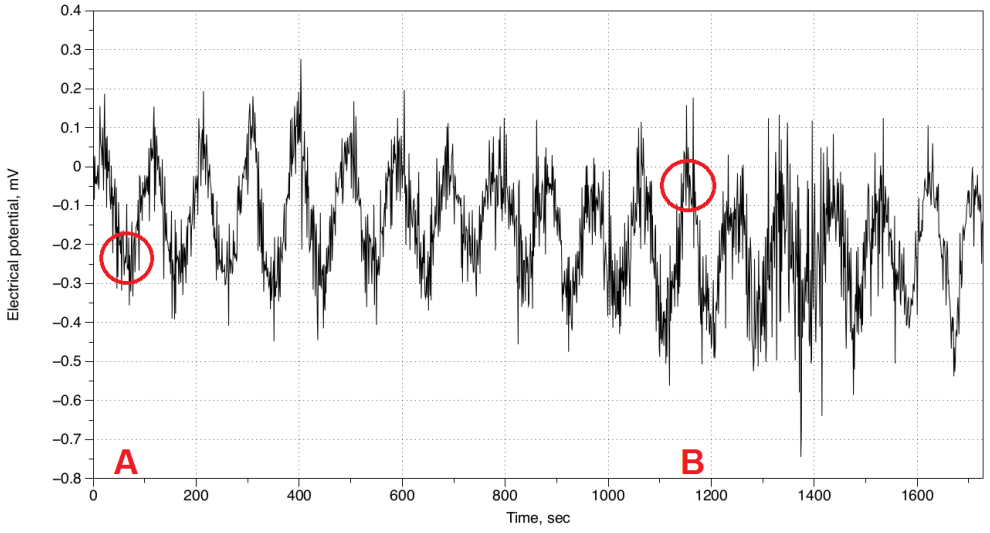
Although technically a single-celled organism, plasmodium's comparatively enormous scale necessitates a markedly different set of cytological features from other unicellular



(a)



(b)



(c)

FIGURE 2.4: Contractions in a plasmodial tubule. (a–b) Photomicrographs of a plasmodial tube taken approximately 1000 s apart with scale bars to illustrate changes in its diameter of nearly 25% (404→494 pixels). (c) Correlative electrophysiological measurement showing oscillation in membrane potential corresponding to vessel diameter. Circled and labelled areas correspond to images [a–b]. Figure adapted from the author's own work in Ref. [201].

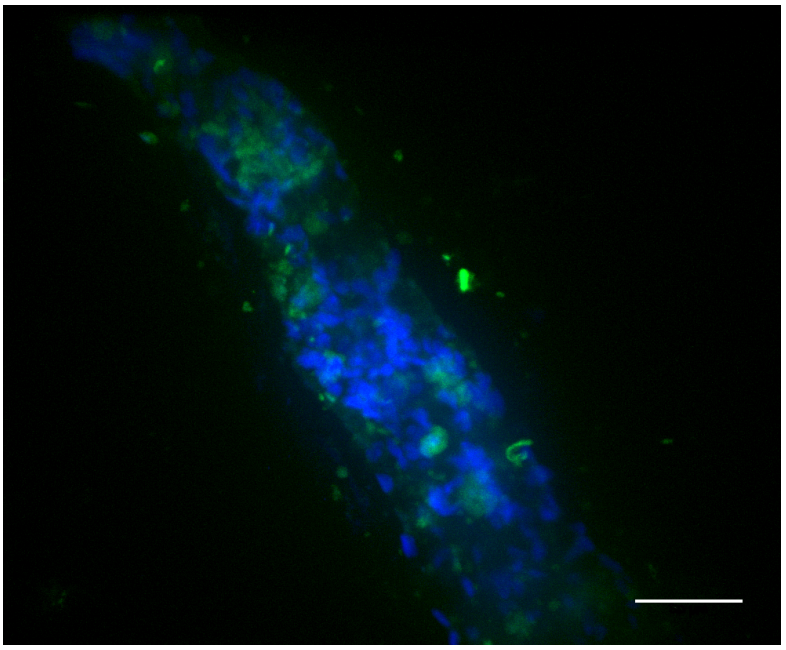


FIGURE 2.5: Confocal micrograph of a small plasmodial tubule. Many nuclei (blue; Hoescht 33342 staining) are visible within the confines of the tube (green; autofluorescence). Figure adapted from the author's own work in Ref. [204].

organisms. For example, in order to facilitate sufficient protein synthesis to sustain the whole organism, each plasmodium may possess in excess of 10^8 nuclei distributed throughout the entire cell (Fig. 2.5), whereas the majority of other more 'conventional' cell types (i.e. animal, plant and fungal) will only possess one [237]. As such, some have suggested that a plasmodium should be regarded as multiple cells living in unison within a single membrane and indeed, they were historically referred to as 'acellular' in order to emphasise this distinction [43]; the modern term to describe this is syncytium, but we refrain from using it here as it may also be used to refer to the cooperative actions of muscle systems within an organ such as the mammalian bladder.

The intracellular architecture of a plasmodium has distinct physiological layers which are conceptually similar to tissue systems in multicellular organisms. In transverse section, a plasmodial tube is composed of three distinct layers (Fig. 2.6):

1. **Slime layer**. Also known as the glycocalyx, this sugar (mucopolysaccharide)-rich exuded fluid sheath from which the slime moulds derive their name serves several purposes, including protecting the organism from desiccation and the effects of ultraviolet (UV) light irradiation[22], solubilising environmental nutrient sources to facilitate their uptake [43, 284] and acting as an extracellular spatial 'memory'. This latter characteristic arises from the organism's tendency to eject its excreta

[22]UV light can be extremely damaging for single cells due to the incidence of direct DNA damage from interaction with photons at this energy level [39].

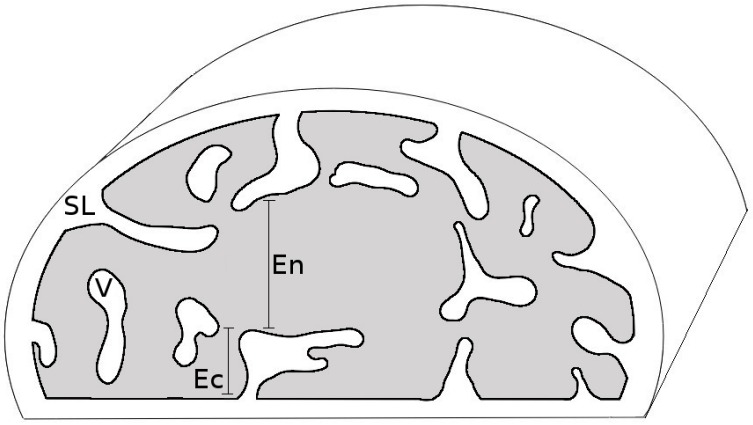


FIGURE 2.6: Schematic diagram of cross-sectional anatomy of a plasmodial tubule. SL = slime layer; V = vacuole, Ec = ectoplasm; En = endoplasm. Darker lines indicate the cell's membrane. Areas shaded in grey represent the interior of the cell. Figure adapted from the author's own work in Ref. [204].

(particles of indigestible nutrients, ions, metabolites etc.) into its slime layer which persists after the organism has migrated away. This mechanism was exploited in one of the first examples of a slime mould computing device; see section 2.3, Ref. [247].

2. **Ectoplasm**. This is a gel-like region that sits about the periphery of the plasmodial tube which is demarcated by the cell's membrane at the border of the slime layer. Cytoplasmic flows are very slow or non-existent in this region, which contains abundant vesicles — phospholipid membrane-bound vessels which carry newly-internalised substances into the cell (endocytosis, or pinocytosis when internalising fluids only), waste substances out of the cell (exocytosis) or storage and transport (transcytosis) of stored compounds — and the presence of the majority of the organism's cytoskeleton.

3. **Endoplasm**. This region constitutes the hydrodynamic (sol) core of the tube which is propelled anteroposteriorly by the force generated by contraction of ectoplasmic actomyosin fibres. Vesicles are significantly less abundant and generally smaller in this region as the majority of endocytotic vesicles merge with the endoplasm contents at the boundary between the two layers, which tends to be indistinct; indeed, ecto- and endoplasm are interchangeable (hypothesised to be dependent on fluid pressure (thixotropy) [232]) and the ratio between the two changes constantly during contraction and relaxation.

As a eukaryote, the *P. polycephalum* plasmodium contains the building blocks that are common to most other forms of life and indeed some have noted that it is a suitable model organism for biomedical research (including toxicity and motility studies) due to

its many similarities to mammalian cells [173]; it possesses, for example, organelles such as ribosomes, golgi bodies and cytoskeleton components which are functionally identical to those found in mammalian, protistic and, to a point, fungi, plant and bacterial cells. With regards to our justifications for using slime mould as a UC substrate, this is encouraging as it implies that the development of a slime mould computer may uncover knowledge relevant to the use of other biological substrates.

Can a slime mould be said to be a 'simpler' organism than any other which we may choose to work with? Are we to say, for example, that unicellularity is equatable with simplicity, perhaps due to the lack of requirement for cooperative cell–cell signalling? Surely not, as various unicellular life forms such as a great many bacterial species communicate with the millions of other such organisms in a colony via a highly complex, non-linear chemical messaging system (quorum sensing) [214]. Communication between different microorganism species (e.g. in biofilms) is furthermore a rich and highly complex topic. We argue that, in essence, there is no such thing as a simple organism, but that *P. polycephalum's* lack of architectural differentiation coupled with its independence from other discrete cells implies that it will be simpler to adapt into a UC device: whilst the organism clearly possesses mechanisms for extracellular sensing and intracellular transduction of environmental stimuli into coherent behaviour patterns, it lacks any real differentiation between other parts of its self as its intracellular environment is homogeneous; it is an amorphous sac of protein and salty fluid, yet it is nevertheless able to function as an independent, discrete unit.

With reference to current knowledge and technological limitations, we conceive that these characteristics make slime mould far better suited to the task of UC than, for example, mammalian cell culture as the organism is not dependent on extensive artificial support in the absence of external structural components (consider that neuron cell culture lacks mechanisms for feeding, detoxification and structural support in the absence of blood vessels, glial cells and connective/supportive tissues) because it is 'simple' in the sense of not being dependent on other cells. This does not imply an advantage over other free-living single-celled organisms such as the infusoria, but these lack slime mould's robustness or ease of cultivation, as was previously discussed.

2.2.5 Basis of Environmental Sensing

As previously intimated, the *P. polycephalum* plasmodium is capable of perceiving attractant chemical sources and coordinating the response to move towards them (positive chemotaxis). At the molecular level, this is achieved through the interactions of environmental chemical molecules with cell membrane-bound receptors or otherwise through

electrostatic interactions with the naked membrane [310, 311]. Both events initiate highly amplifying intracellular signalling cascades (second messengers) which precipitate the behavioural change of instigating directional movement through activation of local motile machinery.

If the reception of a chemical signal leading to its intracellular transduction is regarded as a form of computation as discussed in section 2.1, we begin to realise just how 'massively parallel' an amorphous substrate is. The plasmodial membrane is studded with chemoreceptors and between them, areas of naked membrane are also receptive surfaces: this can be demonstrated by the characteristic radial growth patterns of the organism on a nutrient-rich substrate (Fig. 2.1B), indicating that every aspect of the organism is concurrently sensing a favourable chemical gradient. This equates to an absolutely vast number of individual sensing operations occurring simultaneously; note that, whilst activation of cell surface receptors and consequent activation of second messengers is a process which consumes a certain amount of energy through ATP hydrolysis, there is no centralised control; receptors are not regularly sampled and input data is not ferried to a central control centre for processing. Whilst it would be very difficult to make a quantitative estimate of exactly how efficient this process is, it is clearly an elegant alternative to how sensing is achieved with conventional architectures.

P. polycephalum's responses to unfavourable chemical stimuli are similar except that they result in the organism migrating away from the source (negative chemotaxis). It is unlikely to possess specific receptors for unfavourable compounds [311], hence the repellent effect is believed to originate from membrane interactions precipitating inhibitory or repellent effects on local motive components. In their comprehensive review of *P. polycephalum's* responses to various volatile organic chemicals, De Lacy Costello and Adamatzky [90] found that the organism's migratory responses to environmental chemical sources are proportional to their degree of favourability or harmfulness; oxygenated terpene compounds such as farnesene were strongly attractive and appeared to sustain the organism[23], whereas toxins such as alcohols and aldehydes were amongst the most strongly repellent. Control theory states that the existence of proportional responses to input data implies the existence of a control system which is able to distinguish between stimulus magnitude [3]; although drawing analogies between biological and artificial control systems is a topic too involved to cover here, this does imply the existence of a novel category of 'decentralised and distributed control system' within the organism.

[23]Note, however, that the observance of tactic interactions between an organism and a compound may be coincidental (e.g. many freshwater protozoa such as *Paramecium caudatum* and *Didinium* spp. are attracted to weak acids due to their membrane depolarising effect, rather than because acids are conducive to supporting life; quite the opposite may be true), unless the response is immediately injurious.

P. polycephalum is able to perceive and react to stimulants other than chemical sources, including:

1. **Light**. Possessing at least two varieties of cytoplasmic proteins which enter a high-energy state upon interaction with a photon (photoreceptors) which then catalyse second messenger pathways, *P. polycephalum* is able to perceive and respond to light in a variety of different ways, depending on the wavelength, intensity and duration of exposure [257, 339]. It is photophobic (negative phototaxis) and will generally avoid/migrate away from sources of illumination, although red light (c. 680 nm) has been cited as being an attractant [139, 257]. UV-A (320–400 nm) light may initiate sporulation after prolonged exposure, whereas 540–620 nm light may inhibit sporulation [53].

2. **Physical pressure**. *P. polycephalum* responds to sporadic tactile stimulation with temporary local cessation of shuttle streaming and a concomitant bioelectrical response [22, 35]. This is likely to result from stimulation of membranous stretch receptors which transduce and transmit the stimulus throughout the organism via its actin cytoskeleton in order to spread the pressure and hence minimise local damage [155]. Repeated and continuous tactile stimulation results in the organism migrating away (negative thigmotaxis).

3. **Electrical potential**. The *P. polycephalum* plasmodium will tend to migrate towards the cathode in the presence of a DC field (galvanotaxis) [140]. The organism does not exhibit a specific response to direct electrical innervation. Indeed, the organism seems to be remarkably tolerant to having comparatively large DC currents passed through it [325].

4. **Other forms of electromagnetic radiation**. The plasmodium will migrate along the lines of a magnetic field (magnetotaxis) [95]. Extremely low frequency electromagnetic fields have also been described as retarding streaming frequency, although the mechanisms underlying both of these phenomena are unclear [129, 268].

5. **Temperature**. The plasmodium will preferentially migrate towards temperatures within a preferred range and avoid those outside of it (thermotaxis); the mechanisms underlying this are not understood [334]. Low but non-freezing temperatures will drastically slow cytoplasmic streaming but do not cause deleterious health effects if exposure time is sub-48 hours. Rapid heating will cause cytoplasmic gelation which is reversible if the temperature increase is sub-critical.

This inexhaustive list of stimuli that *P. polycephalum* is able to perceive and react to serves to demonstrate that not only is the organism capable of processing enormous amounts of simultaneous multimodal input, but it is also extremely tolerant to such abuse with regards to its abilities to withstand UV irradiation, electrical stimulation etc. without spontaneously perishing. This further confirms that the organism is an exemplary research organism which should be particularly well suited to adaptation into hybrid artificial-biological UC devices.

2.3 Slime Mould Computing: A Review

We have now seen biological justifications for the use of slime mould as a research organism. We are by no means the first to realise this, however, as 'slime mould computing' is an active research area at the time of writing. This section comprises a literature review of the topic.

2.3.1 Historical Research and First Forays

P. polycephalum research has tended to be a sporadic phenomenon. Although it has generated some significant interest in a great many scientific fields since its first description by Persoon in 1794 [233], the organism has undergone two distinct phases of increased scrutiny during the 20th century in both the 1950s (driven by the works of Kamiya and Abe [166, 168] on the shuttle streaming oscillator and bioelectrical potential which were complementary to Hodgkin and Huxley's ground-breaking advances with giant squid neurons [146]) and 1970s, during which time an extraordinary rise in *P. polycephalum* research occurred (Fig. 2.7) corresponding to the advent of molecular biology and ultrastructural research. Much of the knowledge that current research is based upon was uncovered during these periods; we refer the reader to reviews [44, 333] for historical perspectives on the topic of slime mould research.

The first foray into slime mould computing was, arguably, made by Nakagaki *et al.* in 2000 [219], who reported that the *P. polycephalum* plasmodium could, when placed in a labyrinth, migrate its way towards the exit when guided by a chemoattractant gradient. Furthermore, they found (and elaborated upon in futher publications [218, 220]) that the organism is capable of finding the shortest path out of said labyrinth consistently on its first pass. They characterised this behaviour as an expression of natural computation (implementation of maze-solving algorithms has long-since been an acid test of both computational efficiency — with regards to both graph theory and search algorithms implementing shortest path solutions [266] — and of the viability of

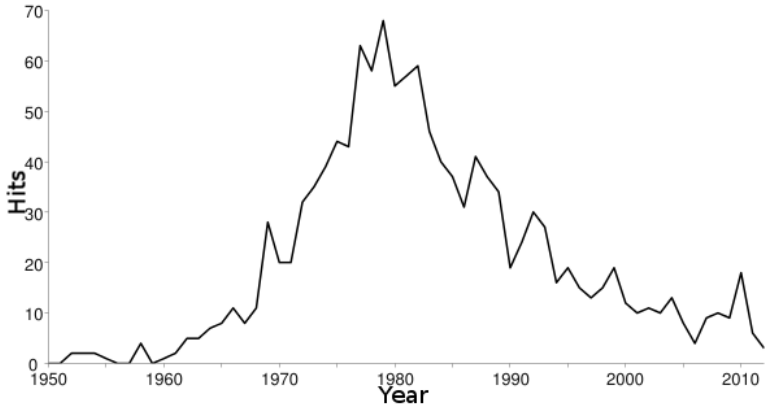


FIGURE 2.7: Graph to show trends in the search term 'Physarum' in the Medline database. Derived from data in Ref. [211].

unconventional computing substrates [26]) as well as a form of what they described as 'primative intelligence'.

2.3.2 The First Decade

The concept of *P. polycephalum* performing well-defined 'computation' — as opposed to the novelty of pseudo-intelligent behaviour — was formalised in 2004 when Tsuda *et al.* [302] demonstrated that the organism's migratory patterns can be engineered to represent Boolean logical operations. Inoculation of one or two plasmodia into geometrically-constrained networks of channels partially-filled with a thin layer of NNA was used to represent input configurations of $\langle 0, 1/1, 0\rangle$ and $\langle 1, 1\rangle$ — i.e. each plasmodium represented an input 'bit' of data. AND, OR and NOT gates were demonstrated by exploiting the organism's preference for migrating towards a nutrient source at the termini of the channel network but repelling down different pathways at points where it met another plasmodium. These gates were slow to operate with propagation delays being essentially limited to the speed of plasmodial migration, but they were found to be moderately robust and also self-repairing when the organism was subjected to harmful stimuli.

In the same year, Aono and Gunji [48] presented their initial thoughts on the development of a novel cellular automata (CA) class (elementary conflictable CA) which they argued was a Turing-computable model of dynamical hyperincursive[24] systems and proposed that slime mould was a material implementation of such a model. Their conclusions were debated by some in future publications, but this nevertheless started the

[24]Hyperincursion is a term coined by Dubois [100] to refer to a hypothetical computing system which is able to display certain emergent features such as anticipation by making reference to an internal mathematical model of reality in order to construct a likely 'future' state. This topic is revisited in Chapter 4.

ball rolling for researchers to begin conceiving of abstract UC schemes involving slime mould.

The latter part of the decade witnessed a gradual increase in the diversity of *P. polycephalum* UC research, which split into the following four topic areas.

2.3.2.1 Graph Theoretical Applications

Inspired by news of *P. polycephalum's* ability to solve maze puzzles, several researchers began to investigate the topological features of the networks generated by the moving plasmodium. In 2008, Adamatzky [12] demonstrated that networks of plasmodial tubes approximate the edges of proximity graphs (defined in Appendix B.3) in experiments where vertices were represented by chemoattractant sources, and evolve their degree of connectivity over time, beginning with minimally-connected graphs (minimal spanning tree) and cycling up to Gabriel graph-levels of connectivity, depending on the favourability of environmental conditions and nutrient availability.

This precipitated the discoveries that the organism is also able to 'compute' the Voronoi diagram and Delaunay Triangulation (defined in Appendix B.3) of any matrix of spatially-distributed attractants and repellents [269, 270]. This was later formalised and built upon by Schumann and Adamatzky [261] who presented a spatio-logical interpretation of plasmodial foraging and tube network formation.

Plasmodial graph theory applications were put to extensive use in demonstrating that the organism could 'design' transport networks in Ref. [32], where the organism was inoculated onto an NNA island cut into the shape of England in the approximate location of London: nutrient sources were distributed at the locations of major cities and the organism was left propagate and link each 'city' with a plasmodial tube. The authors proposed that the organism planned an exceedingly efficient network (using measurements of total node length) and advanced that slime mould could be used by governmental planning departments for real-world transport network planning. This hypothesis would later be rigorously tested through the analysis of current and historical transport networks and various computer models of slime mould propagation (summarised in Ref. [16]).

2.3.2.2 Memory

Perhaps one of the most intriguing conclusions of early *P. polycephalum* UC research was the (perhaps, tongue-in-cheek) reference of Nakagaki *et al.* to the organism possessing some kind of 'intelligence'. This was built upon by Saigusa *et al.* [254] who discovered that when a plasmodium was periodically stimulated with a rapid concurrent

deleterious alteration in temperature and humidity — to which it responded by rapidly slowing its crawling speed — it will exhibit an apparently anticipatory response when stimulation has ceased. Their conclusions were tentative with regards to using the term 'intelligence' and indeed, their supporting dynamical systems model was too non-specific to adequately explain the behaviour they observed, although it was estimated that plasmodial anticipatory responses are, in fact, a by-product of biophysical oscillator (i.e. shuttle streaming and its related processes) coupling. It should be noted here that anticipation in mammals has also been attributed to a function of physiological oscillator coupling [273, 286].

This was expanded upon by Pershin *et al.* [232] who likened the anticipatory response to the phenomenon of memristance (see Appendix B.7) and advanced the theory that the thixotropic nature of the plasmodium's intracellular components provides it with a dynamic 'memory' which modulates the oscillating pressure of cytoplasm in the same way that a memristor alters its resistance based on the load that was last placed across it. With supporting mathematical models, they argued that the intraplasmodial pressure incident upon changing the environmental conditions was sufficient to induce an anticipatory response, i.e. that it is a purely physical phenomenon. When *P. polycephalum* was discovered in 2013 to be electrically memristive [126], the earlier work by Pershin *et al.* was brought under the spotlight once more as it seems unlikely that slime mould intracellular biomolecular and bioelectrical phenomena are entirely independent processes.

2.3.2.3 Decision Making

Much was said in early papers about the organism's ability to 'make decisions'. Whether or not such an organism can be said to 'choose' a course of action is a topic for long and probably unproductive debate — if the organism can be said to be acting automatically or actually calculating which behaviour will be more beneficial based on some internal experiential system is a moot point as the latter implies volition which is, under the Cartesian premise, not possible to demonstrate — but its abilities to react to light stimuli were nevertheless exploited to control a robot [303]. The organism was optically interfaced with a robot whose camera detected changes in plasmodium segment thickness, which was found to decrease in response to local photostimulation provided by either light emitting diodes or environmental sources, and consequently triggered a retreat by the robot. This example demonstrates both the organism's tolerance to utilisation in hybrid circuitry as well as a novel use for its 'decision making' skills (autonomous or otherwise).

2.3.2.4 Theory of Slime Mould Computation

During this period, the computing abilities of the *P. polycephalum* were characterised through both theoretical and experimental works as functioning in a manner consistent with both reaction-diffusion chemical processors [11] and Kolmogorov-Uspensky machines[25] [31]. These works by Adamatzky served to demonstrate the potential for the organism to be adapted into a wide range of abstract devices that, crucially, are suited to general purpose computation: such a device was dubbed a '*Physarum* machine'. This view of slime mould as a malleable, multi-functional UC platform formed the basis of the 'review' of slime mould computing, Ref. [14], and very much influenced the future direction of the field.

2.3.3 Pre-2013

Major discoveries during this period included:

1. **The plasmodium is able to manipulate exogenous substances** [13]. When fed with foodstuffs soaked in food-grade dyes, *P. polycephalum* absorbs the dye and distributes it around the entire cell via shuttle streaming. By inoculating a plasmodium onto a substrate with several different-coloured nutrient sources, the organism was demonstrated to uptake and mix the coloured substances in an entirely programmable manner, concurrently demonstrating its abilities of adaptive transport and expression of migration behaviours characterisable as computation.

2. **Spatial activity is correlated with electrical potential waveforms** [33]. Through multi-electrode measurements of plasmodial membrane potential, the organism's electrophysiological phenomena were demonstrated to reflect its various macroscopic behaviours (propagation, sclerotinization etc.) with characteristic waveforms and frequency modulation. This indicated a direct linkage between the processes observed as computation and bioelectrical activity, thus presenting opportunities for exerting control over the organism through manipulation of these endogenous feedback mechanisms and for electrical interpretation of slime mould behaviour patterns — possibly via a machine–organism interface.

[25] Briefly, this is a class of Turing-complete (runs functions compatible with a conventional Turing machine) computer storage modification machine wherein all data are stored in discrete units that are connected by an undirected graph whose topology may dynamically restructure over time. Data are uniquely labelled and accessible by the read-write head equivalent within a certain neighbourhood about each vertex.

3. **Slime moulds possess an external spatial 'memory'** [247]. Reid *et al.* found that *P. polycephalum* utilises the effluvia trails left behind in the wake of its migration to navigate its environment: by detection of the chemical trace of its spent slime layer, it 'remembers' where it has been and will avoid these areas if it can. This phenomenon was put to practical use in aiding the organism to complete the 'U-shaped barrier test', a navigational task used in the study of both biological and machine behaviour which requires the entity to draw on the topology of its environment to reach an attractant source, as opposed to simple positive taxis.

4. ***P. polycephalum* can compute planar shapes** [20]. After further experimentation into chemical attraction and repulsion [15, 19], Adamatzky found that *P. polycephalum* can compute (assume the form of) various polygonal networks about spatially-distributed matrices of various chemical attractant and repellent sources, including their convex and concave hulls. This further emphasises the ability of the organism for morphological processing of its environment as, coupled with its maze-solving and shortest route-finding abilities, its graph theoretical applications by this point boasted an impressive set of image processing operations.

5. **Slime mould can drive a car** [305]. Following on from earlier experimentation into slime mould robot control, Tsuda *et al.* found that the force generated by plasmodial oscillations in response to optical stimulation can be utilised as a coupling medium for controlling the direction of movement of an autonomous vehicle (a Braitenberg car, which is a conceptual vehicle guided by optical feedback directly coupled to its actuators). This demonstrates that the organism's transport mechanisms are suitable for various control applications in hybrid artificial-biological machinery.

6. **Original slime mould logic gate designs are suitable for cascading** [160]. Jones and Adamatzky simplified designs for slime mould logical gates based on controlled migration in geometrically-constrained environments and advanced designs for combinatorial circuitry, culminating in a binary adder, thus illustrating the viability of slime mould behaviour patterns for carrying out complex logical operations.

2.3.4 Phychip

In early 2013, an international slime mould computing research project was funded by the European Union's 7th Framework Program — 'Physarum Chip (Phychip): Computers from Slime Mould'. The aims of this project, which was conducted by researchers at five European institutions, was to [236]:

> Build functional biomorphic computing devices operated by the slime mould *Physarum polycephalum*. [...] Centred on novel computing substrates, self-assembled and fault-tolerant [slime mould] networks will lead to a revolution in the bio-electronics and computer industry. Combined with conventional electronic components in a hybrid chip, *Physarum* networks will radically improve the performance of digital and analog circuits.

During a three year period, over 100 publications on the topic of slime mould computing were produced and a great many functional slime mould UC prototype devices were fabricated. These advances are summarised in the following sections; the reader should note that the research presented in this document (which principally contributed to the project's deliverables concerning slime mould intracellular computation, morphological computation and the fabrication of hybrid artificial-slime mould devices) was produced during the course of this project by one of its researchers, hence their contributions are not summarised here.

2.3.4.1 Sensing

Development of biological sensing devices is a desirable outcome of UC research as it opens the potential to make 'useful' devices which capitalise upon the favourable characteristics of biological substrates, such as massively parallel sensing. Furthermore, examination of how environmental stimuli are sensed, 'processed' and responded to demonstrates how the intracellular processes which constitute the computation occur. *P. polycephalum's* reactions to chemical and, to a lesser extent, optical stimuli were well characterised by this point, but the extent to which its ability to sense various environmental stimuli could be used towards purposing the organism as a discrete sensing element for use in a multifunctional slime mould computing device was unclear.

After extensive investigations into characterising the organism's response (which was usually via interpretation of migratory patterns and/or membrane potential) to various forms of stimuli — lights of different colours [23], application of physical pressure [22] etc. — a range of discrete computing devices were fabricated on the principles uncovered. For example, a novel type of slime mould tactile sensor was produced by growing the organism over small 3D-printed plastic 'bristles' inserted into NNA hemispheres, whose conformation was designed to mimic the whiskers of animals such as cats and chinchillas, which are purposed for tactile sensing of the animals' immediate environment. The slime mould-colonised bristles were prodded and their electrical response was recorded via underlying electrodes. It was found that these devices were able to accurately interpret stimulation of various durations and intensities.

Multisensorial slime mould devices able to interpret chemical, optical and thermal input and produce a relatively-repeatable, coherent electrical output were later developed [322, 324]. This technique was also used as the basis input/output for a series of logic gates implemented in individual plasmodial tubes (see section 2.3.4.2).

2.3.4.2 Slime Mould Electronics

In 2013, Adamatzky demonstrated [21] that the *P. polycephalum* plasmodium is electrically conductive; though possessing a high electrical resistance in the order of a few mega-Ohms, a single plasmodial tube of 10 mm length was demonstrated to conduct sufficient electricity from an external power supply to illuminate an array of 6 light-emitting diodes (LEDs) for over 24 hours. This was the first instance of a '*Physarum* wire'[26] being used, the design of which was recycled in many consequent publications as a means of standardising slime mould electrophysiological measurements. This design involved growing single plasmodial tubes of relatively constant length (10 mm) by cultivating a plasmodium on a 0.2 ml NNA hemisphere overlying an aluminium tape electrode and siting another hemisphere/electrode loaded with a chemoattractant at a distance of 10mm away. This encouraged the organism to migrate across the gap between the hemispheres to form a single large (approx. 0.3 mm diameter) plasmodial tube between the two (Fig. 2.8). Plasmodial wires are self-routing, regenerate following damage, may grow over a range of substrates if suitable moisture is present (e.g. naked circuit boards) and are tolerant to being insulated with silicon oil; they were accordingly hailed as being one of the first instances of live biomorphic circuitry being used experimentally.

Various attempts were made to enhance the viability of the interface between plasmodial wires and their power supply in order to curtail the organism's high resistance and the inherent resistivity of the NNA hemispheres. Amongst the most successful of these involved growing the organism over layers of conductive polymers such as polyaniline (PANI) and polyethylenedioxythiophene (PEDT); the electrical properties of the interface in these instances produced interesting effects that were demonstrated to have transistive and memristive properties [96, 291].

Another use for plasmodial wires was uncovered following the characterisation of their electrical resistance in resting conditions, which was found to oscillate in antiphase with electrical potential. The mechanisms for this were unclear (although it was suggested

[26]These are henceforth referred to as 'plasmodial wires'; although not the name originally given to them, the author prefers to adhere to biological conventions and not use species names in this manner.

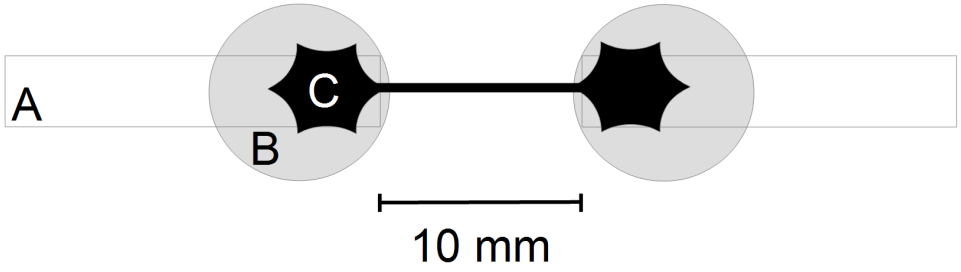


FIGURE 2.8: Schematic diagram for experimental setup to produce a standard '*Physarum* wire'. A = aluminium tape electrode; B = NNA hemisphere; C = plasmodium. Adapted from original design in Ref. [21].

this resulted from alterations in plasmodial tube diameter during contraction) but nevertheless it was demonstrated that plasmodial tubes can be utilised as discrete electrical oscillators [24].

Due to its ability to maintain a dynamic membrane potential, attempts were made to harness *P. polycephalum's* powers of generating an electrical potential in retrofitted microbial fuel cells [294]. Using state-of-the-art fuel cell technology which capitalised on the energy expended by organisms in the oxidation of supplied foodstuffs across a semi-permeable carbon membrane, *P. polycephalum* was found to generate c. 0.5–1.0 V. Although this value was not particularly impressive in comparison to the results obtained from the use of mixed-population colonies harvested from activated sludge and was not supported by any statistics, it was demonstrated that slime mould may act as an adjutant organism in fuel cells already colonised with various species of protozoa.

2.3.4.3 Logic Gates

The logic gate is the fundamental unit of a conventional computer. Although biological substrates are not necessarily well-suited to implementing Boolean logic, a range of devices designed to carry out traditional logical operations were designed during the course of the project in order to demonstrate the viability of slime mould for carrying out general purpose computation in unconventional, heterotic devices. These included:

1. **Microfluidic gates** [35]. Complex networks of plasmodial tubes were allowed to form over a NNA substrate. Specific conformations of tubes with a certain number of interconnections were isolated under a low-power light microscope and were gently prodded with a glass capillary tube. This tactile stimulation caused the local flow of cytoplasm to cease for a short amount of time. The output of these logical gates was interpreted via observing the direction of cytoplasm flow (anterior and posterior flow equating to 0, 1 respectively) in certain pre-designated

'output' tubes at the periphery of the isolated network. Whilst their operation required significant manual intervention, propagation delays for these devices were comparatively fast (in the order of seconds).

2. **Frequency-based gates** [323]. Based on the principles of multisensorial fusion in a plasmodium, 10 mm plasmodial wires were subjected to various forms of stimuli, including light via LEDs and moderate increases in temperature with underlying Peltier elements. The resulting alterations in the frequency of bioelectrical oscillation were interpreted as output, with increases or decreases over a certain threshold representing logical values. Designs for larger-scale logical circuitry such as full adders utilising this technique were advanced but not implemented due to the difficulties inherent in cascading gates of this type, such as long propagation delays (in the order of minutes) and high (>25%) error rates [165].

3. **Combinatorial logic based on plasmodial thermistance** [319]. Walter *et al.* found that plasmodial wires under a load of approximately 0.8 A increase in electrical resistance when heated to approx. 45^{o}C, in essence acting like a thermistor. By connecting three separate plasmodial wire thermistors together in various conformations (along with suitable conventional circuitry to amplify the signal between plasmodia due to the significant voltage drop across them), experimental prototypes of both combinatorial and sequential logical circuits were produced. These devices were very rapid in operation and much more reliable than previous slime mould logic gate designs, with each plasmodial wire being able to withstand tens of duty cycles with apparently no deleterious health effects. Intriguingly, the plasmodial wires were required to be pre-conditioned to the heating stimulus — which was provided by producing the plasmodial wire in a glass tube which had a heating element wrapped around the outside and would rapidly raise the glass tempearture to nearly 70^{o}C — as it would only display such a rapid increase in electrical resistance if it had been previously exposed to such an insulting stimulus at least once. This work therefore lent credence to previous claims of the organism's ability to remember and even anticipate unfavourable events.

2.3.4.4 Logic

A plethora of theoretical works were produced on the logical foundations of slime mould computing. Whilst it is not appropriate to elaborate to any great extent on such a topic in this primarily experimentally-focussed work, achievements of note in the field include development of p-adic valued logic [262], reversible logic (à la Fredkin and Toffoli) [260], slime mould game theory [264] and a slime mould object-oriented programming language [263].

2.3.4.5 Virtual slime mould simulations

Computer modelling is a vital tool in enhancing our understanding of incompletely characterised systems and helps to guide research directions. Jones demonstrated [157–159, 161–163] the ability of a bespoke multi-agent particle model for approximating *P. polycephalum* behaviour patterns, which it does with impressive accuracy. This model employs a diffusive 2D lattice occupied by multiple mobile particles (represented as shaded cells) which react to nutrient sources represented by a generic attractant within: in discrete time, each particle senses local attractant gradients using three offset sensors which are front-facing. The agents rotate to face the sensor with the highest concentration of attractant and migrate towards it at a constant speed. As each cell is vacated in this manner, the agent deposits attractant at the new site; each cell may only hold one particle, so if a particle is unable to move due to its intended location being full, the particle abandons its current move and selects a new random orientation. Because the agents sense and deposit the same attractant an auto-catalytic feedback loop is established, resulting in a complex space of reaction-diffusion patterning. With regard to *P. polycephalum*, the collective position of the agent population represents the structure of the plasmodial network and the movement of agents reflects flux within the plasmodial network. The cooperative dynamics of individual particles modelled with this method have been demonstrated to be extremely effective in modelling emergent phenomena in slime mould such as the self-organised formation and adaptation of the plasmodial network, network coarsening dynamics, pseudopodium extension and withdrawal, establishment and maintenance of shuttle streaming, collective amoeboid movement, and the response to exposure to repellents and light irradiation. Furthermore, virtual plasmodia constructed with this method are able to perform computations more advanced than what has been achieved in laboratory experiments, such as novel forms of plane tessellation, spatial approximation of the Travelling Salesman Problem, data smoothing, path planning, spatially represented approximations of spline curve relaxation, statistical estimation and density classification.

Conversely, slime mould computing has also been used to inspire model design, as demonstrated by Tsompanas *et al.* [301], who developed a 2D cellular automaton impersonation of slime mould migration patterns to aid in the design of wireless sensor networks.

2.4 Summary, Criticisms and Project Methodology

We are now aware of the justifications for UC research and the potential benefits of using biological substrates in UC; slime mould is arguably one of the most promising organisms for this use, at least as a preliminary step towards the use of more 'complex' biological substrates. It is now pertinent to criticise the advances detailed here, as one is prompted to ask how and why they should be built upon: has the thesis presented in section 1.3 not already been demonstrated to some degree and found to be deficient in some areas? We argue that the advances to date in the field suffer from a number of failings, which are summarised here.

1. The majority of laboratory prototypes of slime mould devices with definite practical applications employ the organism as a discrete, passive electrical circuit element. This is unintuitive given what we know of the characteristics of biological substrates, hence future work should focus on exploring the use of slime mould as a carrier of other formats of 'information' (i.e. towards demonstrating polymorphism).

2. Only simple computing tasks have been physically implemented to date despite modelling data indicating that the organism is capable of far more complex — and potentially useful — tasks, such as various forms of image processing.

3. All slime mould device prototypes produced to date capable of logical operations use only traditional Boolean logical schemes (although admittedly they are far from conventional due to their heterotic nature and the dysjunction between input and output data types). This implies that attempts to implement non-Boolean logic should be made; achieving this would indicate polyfunctionality of the substrate beyond the normal operation of a conventional computer.

4. As the *P. polycephalum* plasmodium is an incompletely-defined system, slime mould computer design approaches tend to be top-down, i.e. the organism is viewed as a black box wherein input formats are mixed and macroscopic behaviours are recorded phenomenologically as output. The phenomenological approach essentially ignores the processes that link cause and effect. As these unknown processes are being labelled as computation, future work should focus on manipulation of the intracellular systems that are fully defined, ideally via the organism's inherent feedback mechanisms, to further utilise the resources of the substrate.

5. All prototype devices mentioned here require significant amounts of user time and attention in their operation and output interpretation, thus greatly limiting their practical usefulness. It is currently unknown whether it is feasible to automate

slime mould device operation; whilst this has many potential benefits, it remains to be seen whether this would merely compound errors arising from the variability inherent in the substrate.

Thus, we propose that the range of 'useful' computing tasks currently implemented by *P. polycephalum*-utilising prototype devices is severely restricted to either discrete morphological processors with a limited range of applications or otherwise being a component of a larger device to which a certain amount of the computing is outsourced: hence, they are not polyfunctional. Furthermore, as very little is known about the intracellular processes underlying plasmodial responses to mixed sources of input, we have only a phenomenological understanding of the extent to which they can be called polymorphic.

We aim to address these criticisms, in addition to the points raised with regards to the previously-debated justifications for UC research and the research questions outlined in section 1.3 in the forthcoming chapters in order to advance the field of slime mould computing and hence also biological UC. Crucially, we approach these criticisms by conceptually classifying *Physarum* machines as one of the following three categories:

1. Slime mould heterotic devices (SMHDs), which comprise live slime mould as a key constituent element of a computing device hybridised with conventional electrical hardware.
2. Slime mould morphological processors (SMMPs), wherein the organism's output is interpreted optically as a morphological adaptation of its body in response to input stimuli.
3. Slime mould intracellular processors (SMIPs), wherein output is interpreted on a microscopic scale.

The reasons for this approach are twofold. Firstly, it is apparent that the recent proliferation in slime mould research has led to a lack of well-accepted systematic classification for *Physarum* machines, making it difficult to assess the state of the field directly as various devices exist (theoretical or experimentally-implemented) which have not necessarily been attributed specific purposes. Secondly, it is apparent that the majority of devices produced to date fall within the category of SMHDs, followed by SMMPs; no SMIPs have been demonstrated. Yet, from our analysis of the viability of biological computing substrates, it is clear that some of the most promising attributes of using biological matter for computing purposes lie within the use of these micro-scale processes, as opposed to whole-organism behaviours: it should be noted that, although working at the intracellular level necessarily increases the complexity of any device we create,

the benefits of using slime mould for UC research (ease of cultivation, non-toxicity etc.) counteract this to some degree. Thus, if we are to investigate whether or not slime mould is a polyfunctional computing substrate, as defined in the thesis under assessment, we must demonstrate its viability for creating all three of these device types. One could argue that SMIPs beget SMMPs, and SMHDs may capitalise on mechanisms common to either of these, but nevertheless we find that this classification is at least conceptually helpful.

The structure of the remainder of this document is as follows: Chapters 3–5 examine our investigations into the design, fabrication and evaluation of SMHDs, SMMPs and SMIPs, respectively, and seek to address the research questions previously detailed. Finally, Chapter 6 details the resultant summary of findings and conclusions, in which we discuss the extent to which *Physarum* machines can be used practically and how this knowledge can be applied to the wider field of UC.

Chapter 3

Slime Mould Heterotic Computing Devices

The slime mould heterotic device (SMHD) represents a fundamental link between conventional and unconventional computing technologies. In this chapter, we present the development of an electronics platform for creating reconfigurable SMHDs: in doing so, we demonstrate the organism's polymorphism in its ability to process multimodal input and output stable, repeatable behaviours in a single format — bioelectricity — such that it can be unambiguously interpreted by a semi-automated interface. By extension, we illustrate that this technology may be used to implement a range of devices with practical uses, thereby demonstrating some degree of polyfunctionality in this category of *Physarum* machine.

Initially we demonstrate reprogrammable optically-coupled universal logical gates utilising slime mould as a proof of concept that slime mould behaviour patterns may be recognised as conventional logical operations by an electronic interface. We proceed to present a highly reconfigurable, low-cost digital logic platform with which the *P. polycephalum* plasmodium may be interfaced towards the generation of a wide range of SMHD sensing devices.

3.1 Slime Mould Optically-Coupled Electrical Logic Gates

3.1.1 Introduction

This section details the creation of a new variety of slime mould logic gate which illustrates the relative suitabilities of using light, electricity and plasmodial migration as information-carrying media in a slime mould heterotic device (SMHD). Although using slime mould to implement a single Boolean logical gate is somewhat counter-intuitive given the purported benefits of biological UC substrates, the gates presented here were produced as the first stages of our endeavours to develop a reconfigurable electronics platform for creating SMHDs capable of completing a range of functions incident from multimodal input.

Output interpretation is one of the key limiting features of all slime mould logical gate prototypes produced to date as they have typically relied on the user to optically interpret the organism's behaviour patterns [35, 302] or, in more recently-produced frequency-based gates, manually interpret their electrical output on an oscilloscope [323]. The organism's bioelectrical potential was chosen as the most suitable output format for the devices presented here due to the relative ease and speed of its measurement by conventional hardware (consider that, for example, an optical interface must necessarily be far more complex than a single slime mould logical gate). Furthermore, it was demonstrated by Adamatzky and Jones [33] that the *P. polycephalum* plasmodium responds to various forms of stimuli with characteristic temporary alterations in membrane potential; this equates to a structured output from non-structured sensory input and is therefore a suitable format for interpretation by a conventional electronics interface, as was discussed in Chapter 2.

The gates presented here function on the principle of guiding the growth of a plasmodium with attractive and repellent stimuli between various live electrodes in order to close an output circuit, a live current through which (as the plasmodium is known to conduct electricity in the milliamp range with few or no obvious deleterious health effects (e.g. death, rapid migration away, sporulation/sclerotinization) [21]) corresponded to the device outputting a logical TRUTH. As such, these devices capitalised upon using the organism as both a morphological processor and a physical circuit component.

Light was used as the coupling medium for directing plasmodial migration in these devices as the majority of other slime mould logical circuits to date have illustrated that using other sources of attraction or/and repulsion (usually chemical) suffer from the detriment that inputs cannot be reprogrammed following device initialisation; light, on the other hand, can be dynamically reconfigured. This approach was informed by Ref.

[33], which demonstrated that a migrating plasmodium can be steered with repellent optical stimuli.

3.1.2 Methods

3.1.2.1 Phototaxis experiments

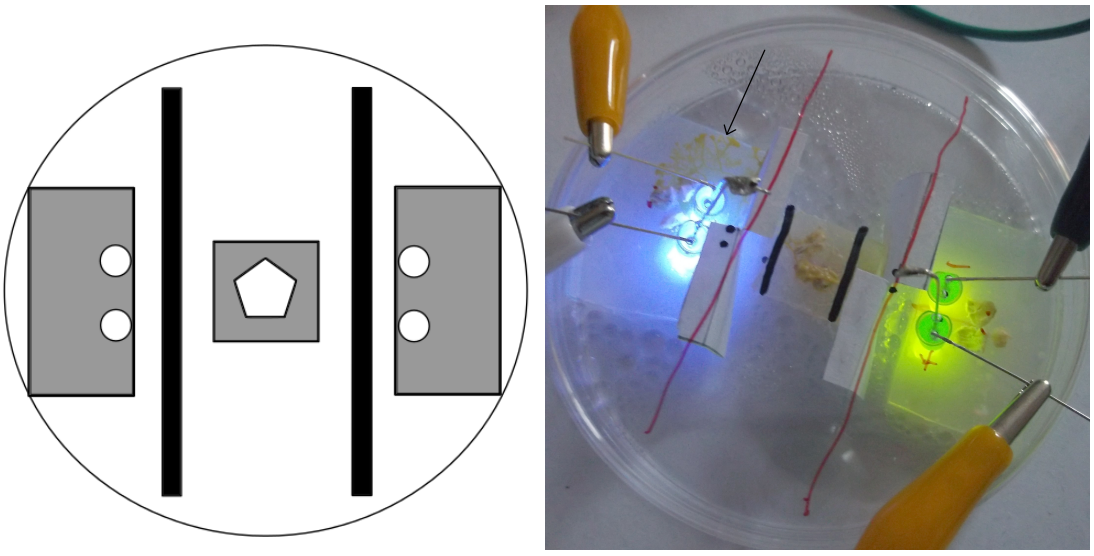
Stock cultures of *P. polycephalum* plasmodia (strain HU554 × HU560) were cultivated on 2% non-nutrient agar (NNA) (Sigma-Aldrich, Germany) in the absence of light at room temperature (22±3^{o}C) and were provided with porridge oats as a nutrient substrate. Subculturing was performed every 3–4 days, as required.

A brief scoping study was performed in order to ascertain which of the following four varieties of low-cost, readily-available LED produced the strongest repellent effects and was hence most appropriate for use in the logic gates under discussion:

1. Blue, λ 466 nm, 50 mCd.
2. Green, λ 568 nm, 40 mCd.
3. Yellow, λ 585 nm, 36 mCd.
4. Red, λ 626 nm, 25 mCd.

These experiments were conducted by mounting 2 arrays of 2 identical LEDS through the lid of a plastic Petri dish, as in Fig. 3.1: plasmodial homogenate was transferred to a small square of 2% NNA in the centre of the dish. Two larger pieces of NNA were situated to the sides of the central square, directly underneath the LED arrays. Both of the peripheral NNA segments were loaded with chemoattractants (3 oat flakes) in order to provide a uniform attractant gradient between them. Thus, the plasmodium was given the choice to migrate towards either of the illuminated areas or stay in its initial position, which was protected from light interference by two opaque cardboard dividers stuck to the lid of the Petri dish leaving a gap of 1 mm for the slime mould to migrate under. All experiments were conducted in the absence of external light sources. Each experiment typically took 3–4 days to complete. Each combination of colours was used and each experiment was run in triplicate. The experimental data for this scoping study are included in appendix C.1.

P. polycephalum's preference for LED illumination by colour is, arranged in order of most → least avoided:



(a) (b)

FIGURE 3.1: Phototaxis experiments to determine the most repellent colour of LED-generated light. (a) Schematic diagram of experimental set-up. Grey squares = NNA; pentagon = slime mould inoculation site; circles = LEDs; black rectangles = cardboard dividers. (b) A completed phototaxis experiment. The plasmodium (arrowed) has migrated to the side illuminated by blue LEDs. Adapted from the author's own work in Ref. [206].

$$\text{Green} \rightarrow \text{Red} \rightarrow \text{Yellow} \rightarrow \text{Blue}$$

Green LEDs were therefore used in logical gate prototypes detailed in the following sections.

3.1.2.2 NAND gate design and function

NAND gates were designed as follows: 3 aluminium tape electrodes measuring $100 \times 8 \times 0.5$ mm, X, Y, Z, were stuck to the base of a plastic Petri dish with a gap of 10 mm separating each of them (Fig. 3.2a). 0.5 ml NNA hemispheres were placed overlying the tip of each electrode: the hemisphere overlying electrode X was inoculated with plasmodial homogenate whereas Y, Z were loaded with an oat flake to encourage plasmodial migration. Electrodes Y, Z both had their own overlying 2-LED array, A, B overlying them, the illumination of which corresponded to the device's input, i.e. if electrode Y was illuminated by array A, the device's input configuration was equal to $A = 1$. A cardboard divider was present between electrodes Y and Z to prevent light contamination between the two. All experiments were conducted in the absence of external light sources.

A	B	Out
0	0	1
0	1	1
1	0	1
1	1	0

TABLE 3.1: Truth table to show the input/output configurations of a NAND gate.

The aluminium tape electrodes were connected to a single output circuit which was connected to a power supply set to a constant 9 V, 0.1 A, values indicated as being appropriate to induce a measurable current in the output circuit without causing deleterious effects to the health of the organism. Thus, when the slime mould at electrode X migrated to either Y or Z, it would complete the output circuit.

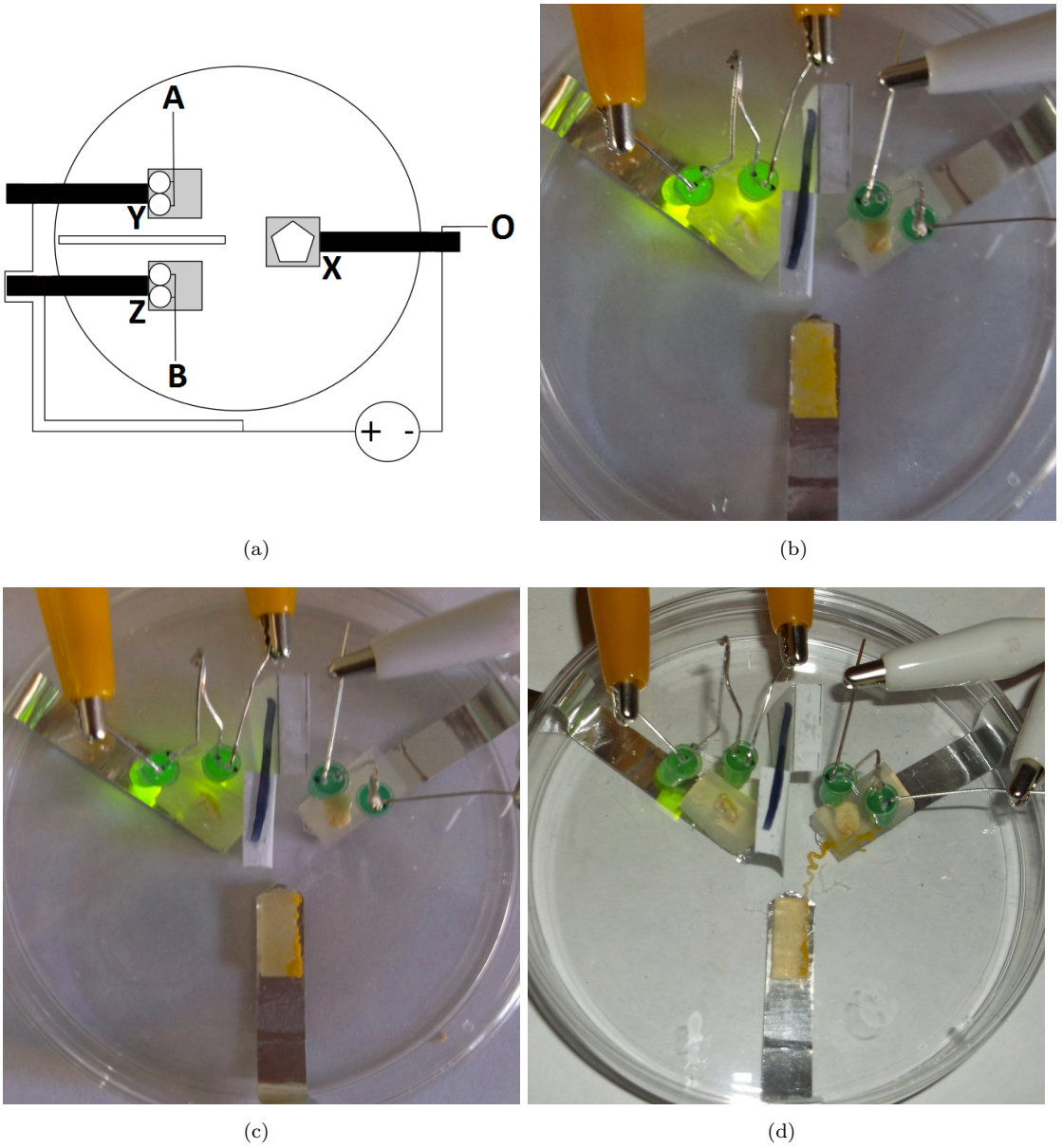
As the purpose of the LED arrays was to repel plasmodial migration, the output of the device was equal to that of a NAND gate (Tab. 3.1): if neither or only one of the two LED arrays were illuminated (input $\langle 0,0\rangle, \langle 1,0\rangle, \langle 0,1\rangle$), the plasmodium was free to migrate to an unilluminated electrode, completing the output circuit and leading to the device outputting a logical TRUTH. If both electrodes were illuminated (input $\langle 1,1\rangle$), however, the organism would be forced to stay at its starting location or otherwise migrate to other regions of the Petri dish, leaving the output circuit open.

3.1.3 Results

Slime mould NAND gates were found to function as expected with every variety of input configuration with a 90% success rate ($n = 10$, where n is number of repeats). An operation for the input configuration $\langle 1,0\rangle$ is shown in Fig. 3.2b–d. Propagation delays were generally within the order of 12–18 h but had a range of 10–22 h. The organism appeared to be very tolerant to having a continuous current passed through it and was even found to tolerate voltages of up to 24 V for many hours at a time in initial experiments, although the intrinsic resistance of the agar hemispheres ($\bar{x} = 18.4$ kΩ, $n = 10$) would have protected the organism somewhat; indeed, the current across the organism was measured as being quite variable but always within the milliamp range.

Gate resetting and reprogramming was attempted through changing the input configuration after an initial operation had been performed. This was achieved at a moderately reproducible rate (median 67% , $n = 3$) for each of the following operations:

1. $\langle 0,0\rangle \rightarrow \langle 0,1\rangle$
2. $\langle 0,1\rangle \rightarrow \langle 0,0\rangle$



(a) (b)

(c) (d)

FIGURE 3.2: Design and operation of slime mould NAND gates. (a) Schematic diagram of gate. Grey squares = NNA; pentagon = slime mould inoculation site; circles = LED array A, B; black rectangles = electrode X, Y, Z; $\oplus$ = power supply. (b–d) Device in operation with input configuration $\langle 1, 0 \rangle$. (b) T = 1 h. (c) T = 6 h. The plasmodium has shifted to the furthest pole of its starting location away from the light source. (d) T = 12 h. The plasmodium has migrated to the unilluminated electrode, completing the output circuit. Adapted from the author's own work in Ref. [206].

3. $\langle 0,1\rangle \rightarrow \langle 1,1\rangle$

4. $\langle 1,1\rangle \rightarrow \langle 0,0\rangle$

5. $\langle 1,1\rangle \rightarrow \langle 0,1\rangle$

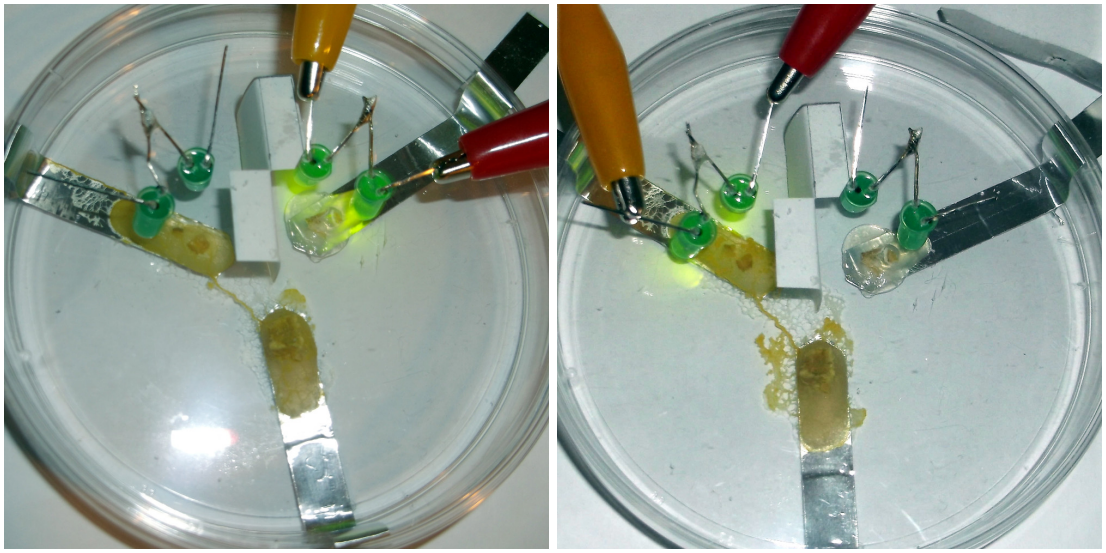
Regarding the fifth operation listed above, the device was reprogrammed soon after the organism had migrated to one random electrode. This is because the organism was highly reluctant to migrate back to an electrode it had previously occupied. Hence, when the device was reset the electrode that hadn't been colonised was unilluminated. This phenomenon of the organism not re-visiting areas it had already explored and vacated prevented resetting of the device. A gate being successfully reprogrammed from input configuration $\langle 1,0\rangle$ to $\langle 0,1\rangle$ is shown in Fig. 3.3.

3.1.4 Discussion

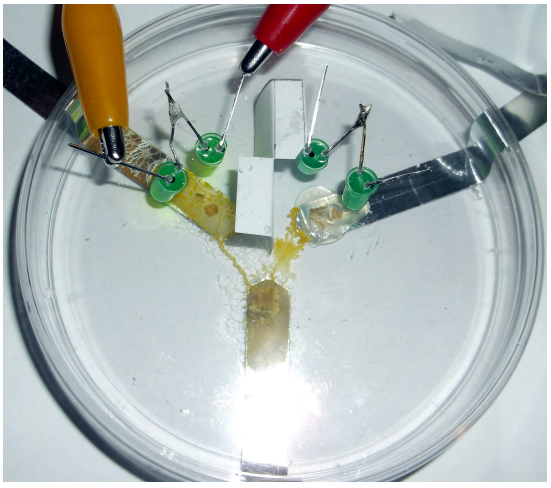
The principle findings of this brief study were that slime mould migratory patterns can be 'programmed' using optical inputs to complete a logical function in hybrid devices containing conventional electronics hardware. Note that whilst we chose to implement a NAND function by virtue of its being a universal logic gate, our methodology could be altered with relative ease to fulfil a range of other logical operations. Although we did not opt to interface these devices directly with a computer, their output was in a form that could readily be interpreted by a wide range of architectures, i.e. binaric electrical current.

P. polycephalum's order of preference to the colours of light used in the phototaxis experiments was somewhat surprising, both from a physical perspective (in that there was no obvious correlation between wavelength or luminous intensity and strength of repulsion) and because historical literature indicates that *P. polycephalum* is most strongly repelled by blue light [257]. This indicates that the organism is likely to possess photoreceptor proteins that are excited by light of circa 568 nm wavelength, the activation of which initiates a withdrawal response.

The gates presented were not without severe detriments which limited their usefulness, with their propagation time of up to one day per operation and unreliability (in comparison to conventional electrical components) being the most significant. As such, although technically functional, these prototypes highlight that any slime mould device built on the principle of plasmodial migration will suffer from a protracted operation time. The prototypes presented here are therefore essentially of no practical use but rather represent a proof of concept that multiple input formats may be used to program repeatable



(a) (b)



(c)

FIGURE 3.3: Resetting and reprogramming a slime mould NAND gate. (a) T = 20 h, following a completed $\langle 1, 0 \rangle$ operation immediately prior to inverting the inputs. (b) T = 36 h. The slime mould has begun to withdraw from the left-hand electrode. (c) T = 42 h. The plasmodium has begun to migrate towards the right-hand electrode and withdraw its tubule linking the left and centre electrodes. Adapated from the author's own work in Ref. [206].

behavioural outputs in the *P. polycephalum* plasmodium; of these, optical and chemical stimuli are viable input formats. Coupled with the demonstration that these multimodal inputs may be used to instigate repeatable output in another format (migration/bioelectrical phenomena), this goes some way to demonstrating polymorphism in the substrate.

In comparison with the slime mould logic gates that preceded those presented here (which were discussed in section 2.3), optically-coupled gates here have a similar accuracy (i.e. frequency of correct completion of the operation) to the forerunner devices presented by Tsuda *et al.* in 2004 [302], which were quoted of having a success rate of "about 85%". Whilst the predecessor gates had a less-variable and somewhat smaller propagation delay than the author's optically-coupled 'second-generation' devices, however, they based on the principle of approximately synchronised growth of two separate plasmodia and hence required significantly more user input to initialise than the gates presented here; their output was also not conducive to being easily interfaced with a conventional computer. Both of these beneficial characteristics possessed by the author's optically-coupled gates represent clear advantages towards creating SMHDs.

The gates presented here also demonstrate that slime mould logical gates are highly tolerant to environmental instability, as evidenced by their relative reliability (from a biological perspective) in spite of an extremely crude environment, and robustness (even in comparison to conventional electronics) to factors such as enormous over-voltage. This is an enthusing finding in context with the aims of creating SMHDs with practical use as well as demonstrating the polyfunctionality of *Physarum* machines. Indeed, a 'third-generation' slime mould logic gate design advanced in 2016 has capitalised upon the organism's noteworthy resistance to various forms of insulting stimuli (electrical stimulation, rapid changes in temperature) whilst concurrently exploiting mechanisms far more rapid than plasmodial migration to achieve combinatorial logic operations, namely the thermistance-based gates presented by Walter *et al.* [319].

3.2 Development of a Slime Mould-Computer Interface

3.2.1 Introduction

To briefly recapitulate the intended role of the SMHD in context with our findings in section 3.1, whilst the SMHD is perhaps the most researched upon variety of slime mould computing device to date, all previous works have focussed on creating single-purpose (monofunctional) devices which are capable of generating input to the organism in only one format. Furthermore, the vast majority of these extant prototypes rely on arduous

manual input instantiation and output interpretation, which severely limits their practical usefulness. This section presents the development of a multi-use platform which enables input/output automation towards addressing these failings, thereby contributing to the overarching investigation into the degree to which the SMHD may exhibit polyfunctionality and/or polymorphism.

The following investigation was carried out in order to develop a slime mould–computer interface according to the following specificiations:

1. Output recognition via a rapid, non-invasive electrical interface.
2. Platform adaptability, such that multiple input formats may be used and different operations can be completed through minor changes to the experimental environment and interface programming.

The interface produced was based on a Cyclone II (Altera, USA) field-programmable gate array (FPGA) board: FPGAs are favoured amongst the research community due to their being dynamically-reconfigurable logic platforms (i.e. they can be programmed to fulfil an extremely wide variety of logical functions at any point), energy efficient and low price in comparison to application-specific integrated circuits [181]. The use of an FPGA does however present the obstacle that such architectures are only capable of receiving digital electrical input, whereas the *P. polycephalum* plasmodium's membrane potential is an analogue system. This was overcome by digitising the measured electrophysiological data with an analogue-to-digital converter (ADC) circuit.

In order to demonstrate the efficacy of the interface, we present a basic but fully-functional SMHD sensing device. The use of light or chemical input was decided against in these first-generation devices in favour of an input format that causes an immediate response from the organism, namely tactile stimulation, in order to reduce the complexity of the task of testing the interface. As was mentioned in section 2.2.5, the plasmodium responds to the application of physical pressure by temporarily ceasing local shuttle streaming and produces a characteristic spike in membrane potential; this was used as the basis for the slime mould bristle sensor [22].

3.2.2 Methods

Stock plasmodia were cultivated as per the methods in section 3.1.2.1. Plasmodial wires were then produced as per the protocol outlined in section 2.3.4.2 and Ref. [21] (Fig. 3.4); single plasmodial tubes were used rather than complex tube networks in order to ensure that only a single oscillatory waveform was being output by the slime

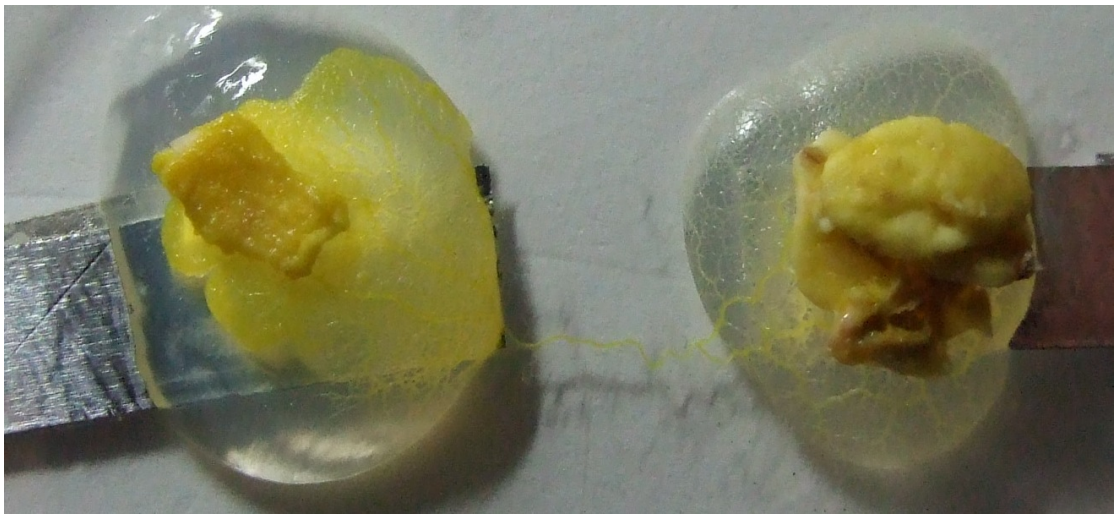
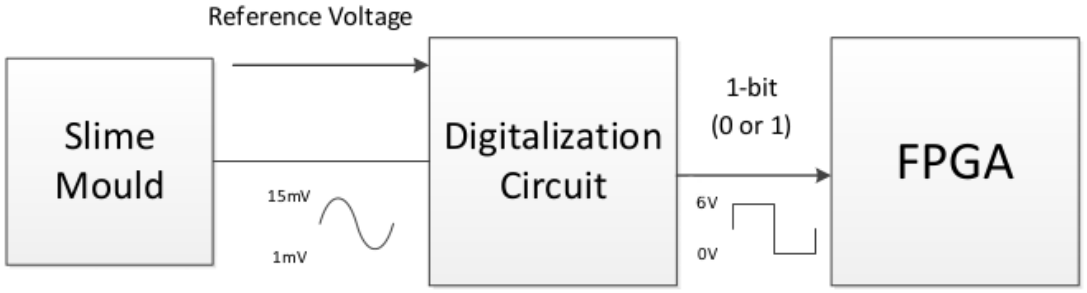


FIGURE 3.4: Photograph of a plasmodial wire spanning two agar hemispheres overlying aluminium tape electrodes. Adapted from the author's own work in Ref. [209].

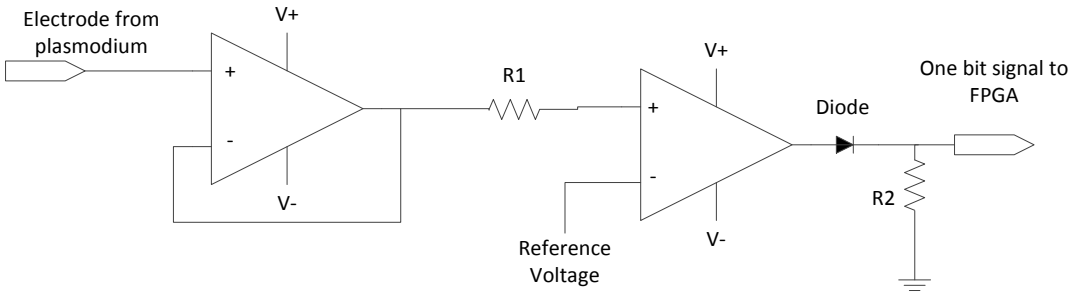
mould element of the device, even in the event that significant morphological adaptation occurred mid-operation.

Each slime mould–FPGA interface consisted of a plasmodial wire connected to the FPGA via a separate bespoke ADC circuit which was fed with an external power supply, as per the box diagram in Fig. 3.5a. The ADC circuit consisted of two operational amplifiers (op-amps): the first was used as a unity gain amplifier (voltage follower) whose purpose was to isolate the input analogue voltage from the plasmodial wire from interference generated within the rest of the circuit by virtue of its extremely high input impedence. The second op-amp was used as a voltage comparator, the function of which was to compare the input voltage from the follower to a reference voltage. The reference voltage value was set with a sensitive 25-turn rotary potentiometer in order that the comparator output a high signal, 6 V (i.e. a digital '1' output), if the measured input value exceeded that of the reference voltage and *vice versa*. As the output of the comparator is the inverse of the high signal, −6 V, when in a low state resulting from the input voltage being smaller than the reference value, a diode was inserted between the comparator's output and the FPGA pin which renders the final output of the ADC circuit as 0 V in such an instance. Thus, the ADC outputs 6 V (1) or 0 V (0), essentially performing a 1-bit quantization on the input signal, which is then forwarded to an input pin on the FPGA. The wiring diagram of the ADC is shown in Fig. 3.5b.

The FPGA was programmed to perform three basic arithmetical operations: counting, addition and multiplication. FPGA modes were programmed and initiated via a software link (Nios II, Altera, USA) with a personal computer. After being programmed, the FPGA may then function without a software link if fed with a 9 V supply or a battery. The counting function initiates a tally whose value increases by 1 for every millisecond



(a)



(b)

FIGURE 3.5: Schematic diagrams to represent the structure of the slime mould–FPGA interface. (a) Block diagram of the system. (b) Wiring diagram of the external ADC circuit. Adapted from the author's own work in Ref. [209].

that an enable signal ('1') from the ADC is present. The count is visible via the FPGA's software interface and also on a 4-digit display on the board (Fig. 3.6). For the addition and multiplication functions, the FPGA either adds or multiplies an operand derived from the plasmodial wire and one from the user via the FPGA's on-board switches; the plasmodium-derived operand was acquired through sampling the input from the organism over three sampling windows, $\langle a, b, c \rangle$, the period between which was set at 50 s (this duration was selected as an appropriate time for the organism to recover between stimuli following scoping experiments), i.e. sample a at $t = 0$ s to derive the first operand, b at $t = 50$ s for the second operand and c at $t = 100$ s for the final operand. The FPGA therefore collects 3 bits of information from the plasmodium over 100 s which it converts into a binary number, the minimum and maximum numbers of which are defined in Eq. 3.1.

$$\begin{aligned} min[(a \times 2^0) + (b \times 2^1) + (c \times 2^2)] &= 0 \\ max[(a \times 2^0) + (b \times 2^1) + (c \times 2^2)] &= 7 \end{aligned} \tag{3.1}$$

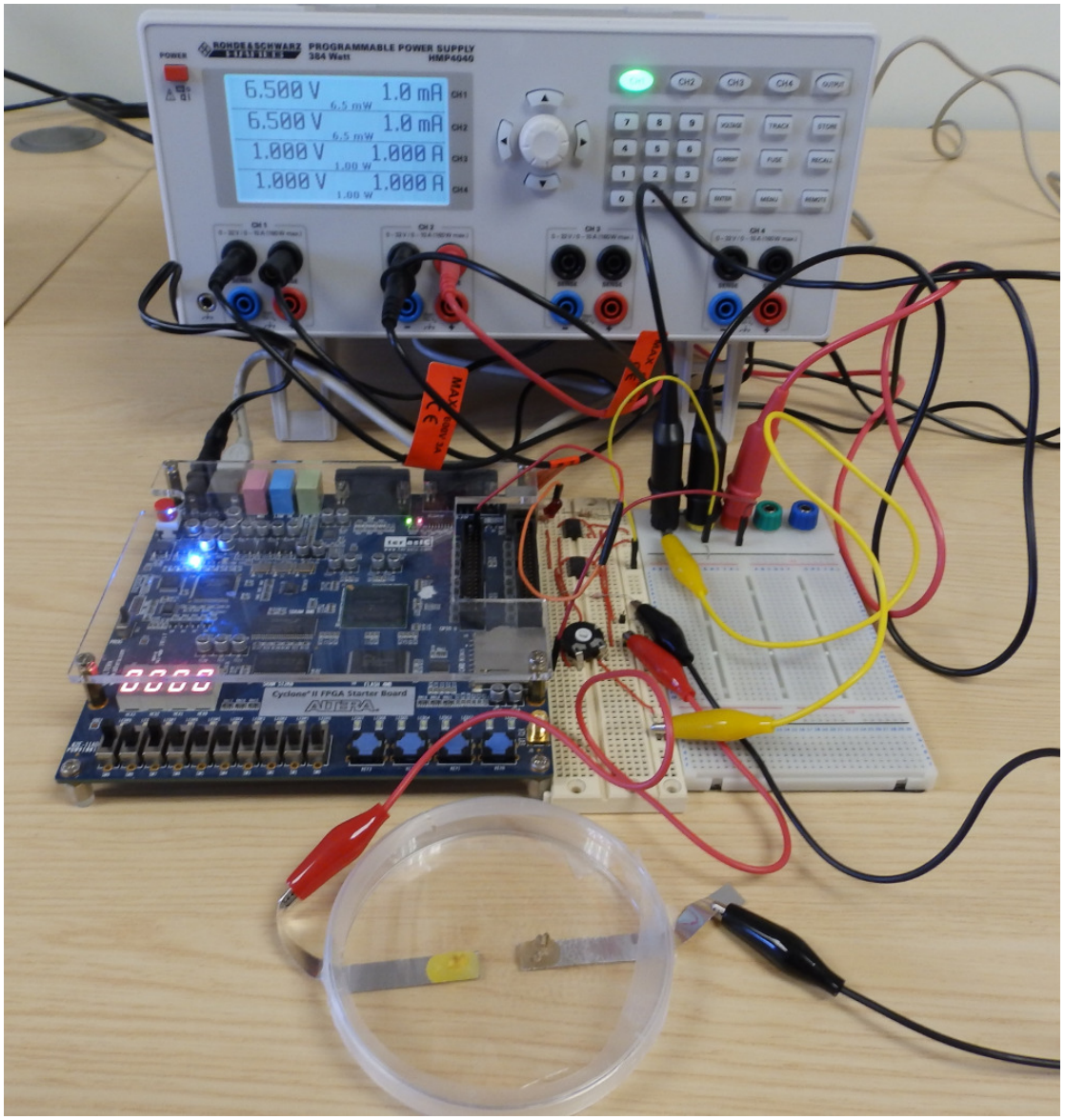


FIGURE 3.6: Photograph of the slime mould–FPGA interface. Note the FPGA's 4-digit display reading '0000'. Adapted from the author's own work in Ref. [209].

For example, the input configuration $\langle a, b \rangle$ would produce '011', i.e. 3. This value is then added to or multiplied by the user-defined operand and the output is displayed via the FPGA's 4-digit display as well as its software link.

The experiments performed in order to assess the device's functionality were as follows: plasmodial wires were connected to an oscilloscope for 300 s; those that were not electrically oscillating were assumed to be in a non-resting state and were not used in consequent experiments. Of the plasmodial wires that were in a resting state, their peak amplitude A and period P of oscillation were measured before being connected to the ADC circuit. The counting function was assessed by setting the reference voltage to the mean membrane potential of the oscillating plasmodial wire such that the counter would,

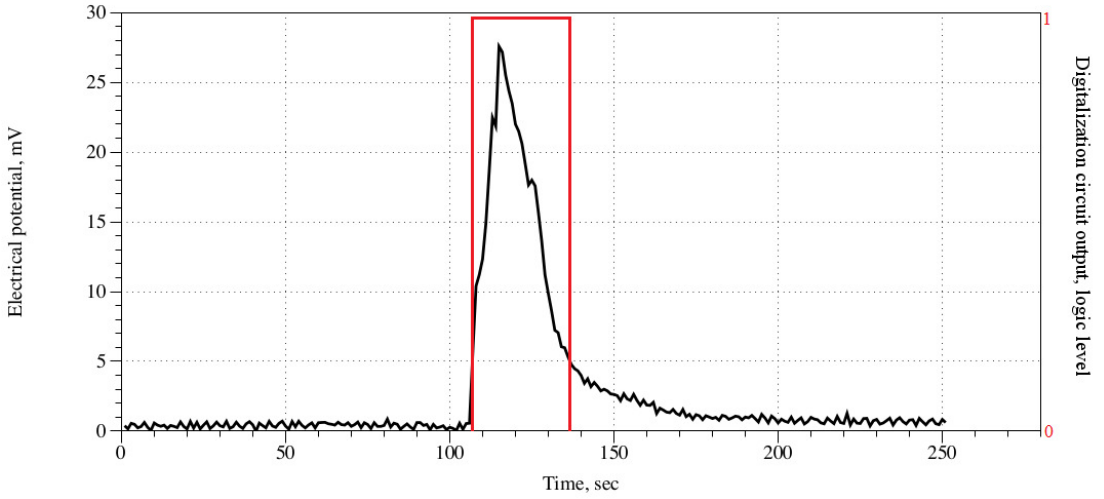


FIGURE 3.7: Graph to show a typical bioelectrical response to a plasmodial wire having a 0.1 g mass placed across it. The plasmodium was stimulated at 105 s, causing a 28 mV spike which persists for a short period but drops to below the threshold value before the next sampling window 50 s after the initial stimulation. The ADC output is overlaid in red. Adapted from the author's own work in Ref. [209].

if functioning correctly, only count for half the duration of P, i.e. for the half-wave when the oscillating membrane potential exceeded the reference voltage. The counter value was collected over five successive periods of counting and its mean value (i.e. counter value $\div$ 5) was compared to the calculated value of the organism's streaming period.

The addition and multiplication functions were investigated by implementing a basic tactile sensor. This was achieved by setting the reference voltage to a value higher than A such that the ADC would only generate an enable signal when plasmodial membrane potential exhibited spiking behaviour incident of tactile stimulation. Initial experimentation revealed that the organism's response to tactile stimulation by laying a 0.1 g glass capillary tube perpendicularly across the centre of the plasmodial wire was to invoke a spike in membrane potential significantly higher than A (mean 27.4 mV, range 19.2–31.5 mV, $n = 5$), which typically receded back into normal oscillation patterns in about 40 s. The glass capillary was left *in situ* until the end of the experiment. Hence, the reference voltage was set to 5 mV higher than A and the sampling window was set to 50 s. A typical electrical response from a plasmodial wire to tactile stimulation is shown in Fig. 3.7.

All experiments were repeated 10 times.

3.2.3 Results

All functions were demonstrated to work as intended. The counter function was observed to start and stop rhythmically with time periods approximately equal to $0.5P$, although

these values were not significant with results typically lying within a ±10 s range of the measured value. The counter was observed to start and stop spasmodically in phases of transition between periods of solid counting or cessation which typically lasted for several seconds.

Addition and multiplication functions were run at 90% and 80% success rates, respectively, using randomly-generated operands for both user and slime mould-derived values.

3.2.4 Discussion

The devices presented demonstrate only one-way communication between computer and organism; indeed, the plasmodial wire could be regarded as carrying out very little, if any, computation in its role as a dynamic sensing element. What we have presented, however, is a functional SMHD built upon a basic but nevertheless highly adaptable, low-cost development platform for SMHDs. Adaptation of automated input, such as optical stimulation via LEDs or thermal stimulation via Peltier elements controlled by the FPGA, is a clear and relatively simple direction for further development of the interface.

The low cost, ease of operation/programming and feasibility of implementing automatic control of multimodal input/interpretation of output this interface offers are its key advantages in comparison to the first slime mould-computer interface (mentioned in section 2.3) fabricated by Tsuda *et al.*, which was an optical interface for enabling slime mould control of a robot's movement [303, 305]: whilst the latter is no doubt ingenious, the complexity of an interface capable of machine vision necessitates much higher monetary and operator time costs to fabricate and operate than the FPGA-based solution presented here.

Indeed, it is worth noting that the FPGA-based slime mould interface may also be suitable for use with other varieties of excitable cell (following minor alterations to its experimental environment, components and programming), as current technologies for cell-computer interfaces such as multi-electrode arrays (MEAs) are typically extremely expensive[1], complex devices tied to only a few specific functions by proprietary software. The build quality and measurement accuracy of such proprietary are, of course, likely far superior to open FPGAs fed by signals routed through open breadboards, hence we propose that comparing the two types of system across a range of cell types and applications is a profitable route for further study.

[1] As of 2016, a basic MEA system for use with neural cell culture costs in the region of £20,000 [217]; compare this to the total system cost of the FPGA board and components listed here which totalled less than £300.

With regards to the feasibility of using the platform for further experimentation, it must be emphasised that there were inaccuracies in some of the results obtained such as the intermittent enable signal observed in the counter function and the sub-100% success rate of counting and multiplication functions. The experiments designed to test the counter function, for example, do not factor in the natural minute-by-minute variations in shuttle streaming period. Whilst these issues further highlight the variability of living systems in comparison to the electronic systems we wish to hybridise them with, they are not insurmountable through minor adaptations to experimental techniques and the hardware used, such as migrating from an open electrode/breadboard system to a bespoke solid-soldered interface in efforts to reduce measurement inaccuracy.

To address the most pertinent criticisms of the devices presented, using single plasmodial wire devices limits the capability of any derivative device to 1-bit operations. Future work should therefore focus on multi-electrode interfaces which measure responses from multiple points in multi-tubule/wire networks. Additional areas for further study include:

1. Experimentation into using further forms of plasmodial stimulation, as well as multi-sensorial fusion.
2. Longer duration experiments in order to assess the stability of devices over time, as maintaining signal integrity when morphological adaptation eventually occurs will likely require a multi-electrode environment and signal multiplexing.
3. Development of computer recognition of plasmodial signals, as well as automated stimulation as discussed.

Crucially, we propose that the prototype system presented here illustrates the viability of SMHDs as devices with demonstrable practical use, as with only moderate refinement as per the aforementioned recommendations a wide range of functions could be implemented. For example, a light-based slime mould–FPGA interface may find use in applications such as — to pick entirely arbitrary examples — power-supply switching between solar and alternative sources in buildings/cars. This prototype therefore demonstrates how SMHDs may be adapted for sensing purposes, thereby illustrating some degree of polyfunctionality.

Finally, although the SMHD promises a far wider and more useful range of uses than the individual logical gates that had been previously produced, they are still not without their detriments and the practical applications of fully-developed SMHDs are admittedly niche. This is discussed further in context with the overarching aims of this document in Chapter 6.

Chapter 4

Morphological Processing With Slime Mould

In this chapter, we contribute to the development of slime mould morphological processors (SMMPs) through optical characterisation of the organism's macroscopic responses to both attractive and repellent stimuli. In doing so, we demonstrate that the organism is capable of what can arguably be called a range of more complex computational tasks than has been previously achieved within the realm of SMMPs, hence increasing the potential functionality of the *Physarum* machine. The results obtained further demonstrate that unstructured sensory input is coordinated into repeatable output by the organism in a manner characterisable as computation. As secondary findings, our results verify certain slime mould simulation techniques whilst providing evidence against an accepted model of plasmodial biophysics.

Initially, we present an investigation into the organism's ability to process images represented by environmental attractant and repellent gradients, after which we investigate applications for the organism's ability to 'memorise' the period of regular adverse stimulation.

4.1 Environment as Image: SMMP Image Processing and Model Verification

4.1.1 Introduction

As was discussed in section 2.3, the slime mould morphological processor (SMMP) is a device wherein the constituent slime mould adapts the morphology of its body shape to sub-divide its environment with an optimised planar graph of plasmodial tubes which link spatially-distributed sources; output is interpreted optically by the user, usually as some variety of proximity graph, the interconnectedness of which is dependent on various environmental and physiological factors. This class of device has demonstrated its usefulness within a particular niche, namely transport network planning, and computer simulations have indicated that they may be capable solving a wider range of graph theoretical problems (e.g. solving the Travelling Salesman Problem [162]).

These successes in the development of the SMMP are significant as space partitioning is an important technique in fields such as computer graphics and machine vision [38], but as these operations only represent a small facet of mathematical image processing, it is pertinent to question whether further uses may be found for this class of *Physarum* machine. This seems likely, as simulations based on the multi-agent particle model of slime mould migratory dynamics developed by Jones (see section 2.3.4.5) have suggested that the organism may be able to represent the shape of a dataset and may therefore be capable of a form of morphological image processing if incited to undergo morphological adaptation in response to environmental stressors [158, 163]. To formalise this concept as a research question: can a SMMP represent the shape (or a function of, therein) of its environment rather than just subdivide it, and by extension can we vastly increase the range and scope of functionality of SMMPs?

To address the basis of this behaviour, although it is unlikely we will ever fully appreciate how the *P. polycephalum* plasmodium 'sees' the world, it is known to concurrently sense both attractants and repellents in its environments as gradients, so will by extension interpret its immediate environment as overlapping analogue fields of net attraction and repulsion [27]. Manipulation of these gradients (or the organism's ability to perceive them) therefore forms the basis of the tools available for programming a SMMP. Note how this is markedly different from the way that conventional architectures interpret a problem such as space subdivision, which is usually based on the principle of dividing space into non-overlapping portions [2]; this is an interesting perspective as the world is of course an analogue space. This implies that the way in which slime mould interprets

an 'image'[1] (as mediated by environmental stimuli) is somewhat more 'realistic' than the methods employed by a conventional computer, which involve discretising the area.

In this section, we describe a series of laboratory experiments demonstrating that the SMMP is indeed capable of computing the approximate solutions to various image processing operations, most notably the skeleton, convex hull and overall shape of datasets represented by attractive chemical and repulsive optical inputs. These operations are defined in the following paragraphs.

A conventional computer will calculate the morphological skeleton of an image as a planar graph derived from morphological thinning (reduction in the size of objects in a binary image through subtraction of shaded cells if they have a certain number, normally > 2, of shaded neighbouring cells[2]) of a particular shape. The resulting object maintains the general shape and connectivity of the original in a manner reminiscent of the way in which an animal's skeleton approximates its body shape, hence the name [89][3]. In plainer terms, the skeleton approximates the shape of an object about a calculated centreline, as is demonstrated in Fig. 4.1a–b. There are many ways of computing the skeleton and each will typically give slightly different results, depending on the size of sampling areas (structuring elements) and algorithms used.

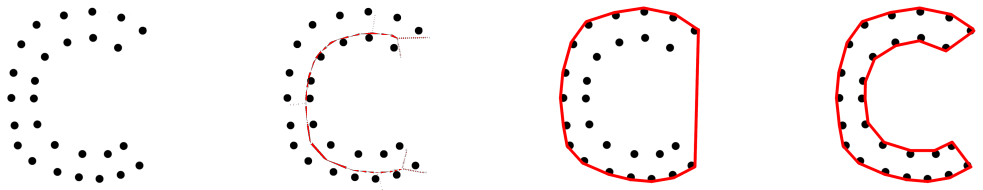
A hull is a shape which encompasses all of the vertices of a graph. The convex hull of a set of points A in a 2D Euclidean plane is defined as the intersection of half-planes to contain A [89]. As we will not be exploring their formalisation by computers in any great depth (although the reader should refer to Refs. [50, 89, 178] for their mathematical characterisation and descriptions of efficient algorithms for their construction), it is pertinent instead to define their construction with a stock analogy: the convex hull of, for example, a series of nails (representing vertices) hammered into a board can be found by stretching an elastic band around the entire set and allowing it to recoil. The concave hull similarly contains a set of points but does so such that it has the minimum possible internal area [102]. The differences between these two types of hull described are illustrated in Fig. 4.1a,c–d.

These morphological operations have extensive uses in computer vision, pattern recognition and various forms of image extraction (e.g. from geographical datasets) [89, 267]. Thus, we may hypothesise that the implementation of such functions with slime mould

[1] This term is included in inverted commas here to denote that we may ascribe the shape of an attractant/repellent gradient as forming a particular picture, but the slime mould cannot be said to interpret it as such.

[2] Note that this method preserves the topology but not geometry of the original shape; methods exist for preserving both [267], although it is beyond the scope of this investigation to elaborate here.

[3] Historically, computation of the morphological skeleton was used as means for inspiring methods of computation based on the properties of natural systems, such as in Blum's grassfire transform [61].



(a) (b) (c) (d)

FIGURE 4.1: Diagrams to illustrate various morphological operations (red) about an array of points (black) in 2D space. (a) Vertices in an approximated 'C' shape. (b) Skeleton of the solid geometric object delineated by vertices in [a], calculated using the GIMP morphological operators plugin [120]. (c) Convex hull, manually constructed. (d) Concave hull, manually constructed.

will lead to a range of further practical uses for SMMPs extending far beyond the realm of subdivision of 2D planes.

4.1.2 Methods

4.1.2.1 Laboratory Experiments

Stock *P. polycpehalum* plasmodia were cultivated as per section 3.1.2.1. Experimental plasmodia were inoculated into 9 cm Petri dishes also containing 2% NNA on which spatial data was represented with the following two sources of input:

1. **Chemoattractants**. Oat flakes were arranged into either specific shapes or regular 10×10 mm grids overlying a NNA layer.
2. **Photorepellents**. All Petri dishes (except for controls) were stored in an environment illuminated by a 7 W array of 24×48 5500 K ('daylight white') LEDs which collectively output approximately 156 Cd. Opaque masks were placed on the lids of all Petri dishes such that the shape of the mask was projected as an area of shade onto the underlying NNA, thus creating non-repellent zones.

The opaque masks stuck to Petri dish lids were cut into the shapes of either the letter 'C' or 'H'; these choices of shape were essentially arbitrary but distinguishing between convex and concave hulls is simple for both. For completeness, the skeleton and hulls of the letter 'H' are shown in Fig. 4.2. In the experiments where oat flakes were arranged into specific shapes, these corresponded to the conformation and size of the mask such that all oat flakes were kept in the shaded region (henceforth referred to as **double image experiments**). The same masks were also used in experiments where the oats were

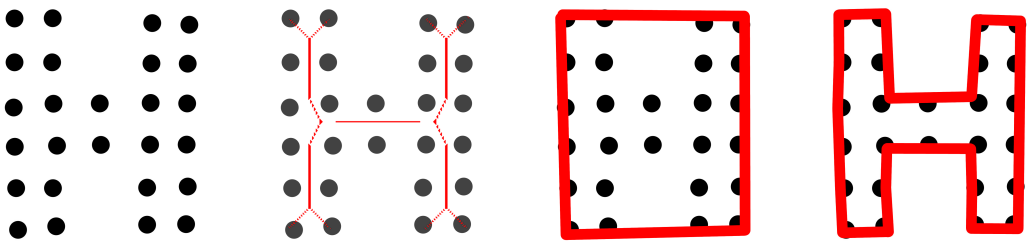


FIGURE 4.2: Diagrams to illustrate various morphological operations (red) about an array of points (black) in 2D space. (a) Vertices arranged in an approximated 'H' shape. (b) Skeleton of the solid geometric object delineated by vertices in [a], calculated using the GIMP morphological operators plugin [120]. (c) Convex hull, manually constructed. (d) Concave hull, manually constructed.

arranged as a grid (**single image experiments**). Control experiments were identical to single/double image experiments, except that no light source was present.

Thus, in control experiments, one would expect the organism to colonise the entire plate and connect all chemoattractant sources with a dense network of plasmodial tubes. The single image experiments were designed to assess the organism's ability to represent shapes mediated by repellent stimuli only; use of a grid of oat flakes sub-divides the space such that it is visually more reminiscent of an array of pixels whilst providing a (relatively) uniformly habitable substrate. The double image experiments existed to discover how the organism is able to represent shapes represented by both attractive and repellent gradients. All experiments were performed in triplicate.

All assessments of image processing by the SMMP under investigation were performed qualitatively: whilst we concede that true image processing is a quantifiable process and that it would not be outside the realms of possibility to perform such analyses here (e.g. subtract pixel values of successive images from one taken at t=0 in order to quantify the amount of growth area under/outside the mask), practical considerations arising from reducing the variability between successive images (camera position, ambient lighting, photograph compression artefacts, variability in NNA layer opacity/-consistency etc.) would render this approach extremely error prone. In light of this and making concessions for the purpose of the investigation, which is to assess the SMMP's ability to approximate image transformations in comparison to both conventional architectures and a virtual slime mould simulation, the authors opted for the qualitative method based on the accepted methods of previous publications on the topic, see Refs. [12, 16, 32, 269, 270].

Images were processed to highlight the structure of plasmodial tube networks using a Processing sketch which isolated and highlighted user-defined colours. These are included for illustrative purposes only.

4.1.2.2 Modelling Experiments

After the laboratory experiments had been completed, attempts were made to replicate the behaviour patterns observed with the multi-agent particle model described in section 2.3.4.5. Briefly, the interaction environment was initialised to mimic those of the laboratory experiments; attractant gradients were represented by a square array of discrete attractant gradients (corresponding to the oat flake array). Repellent fields were represented by grey shaded areas and implemented by damping particle sensing trails by a factor of 0.1 in irradiated areas.

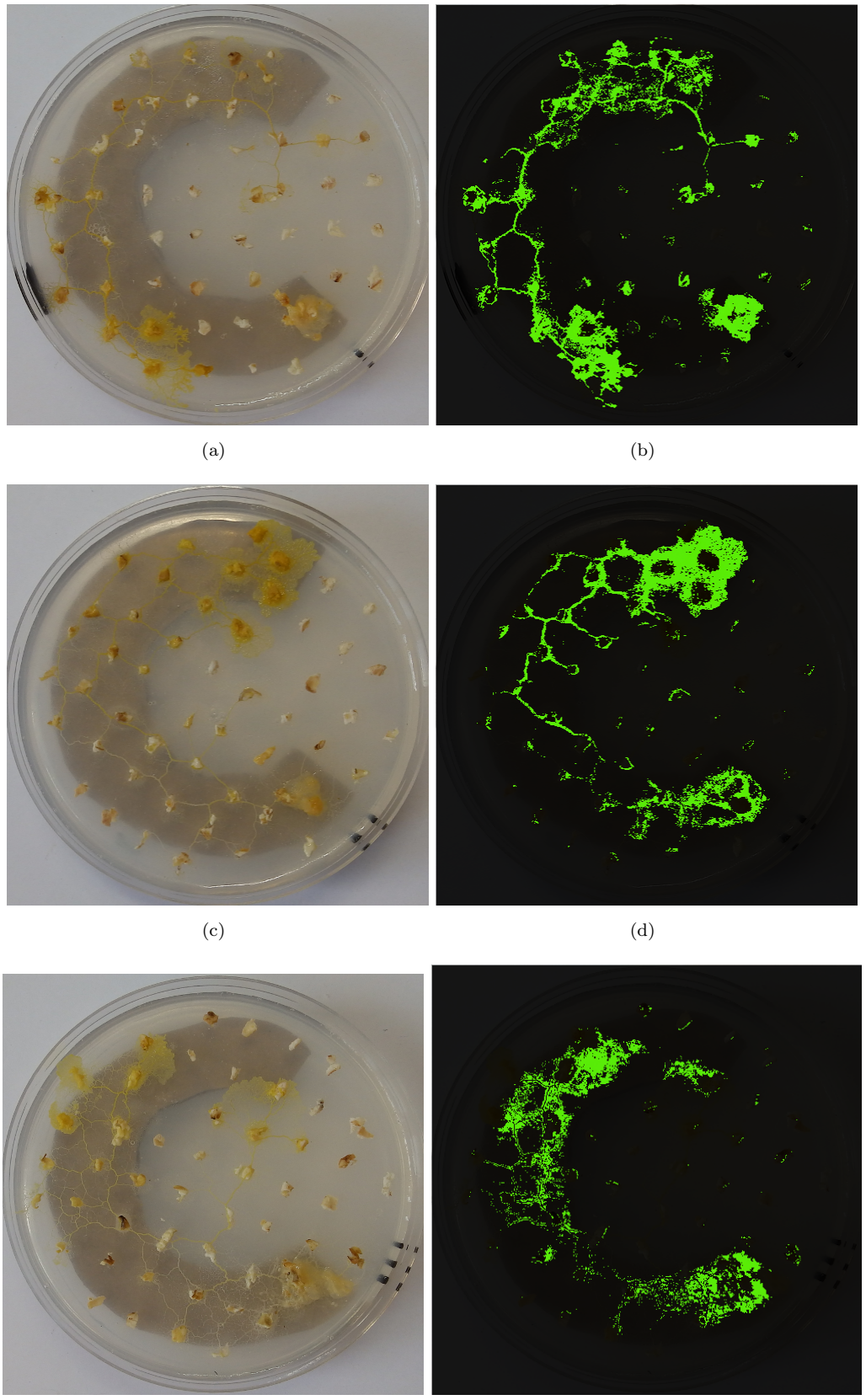
4.1.3 Laboratory Experimental Results and Discussion

4.1.3.1 Single image experiments

Plasmodia were found to propagate principally within shaded areas (Fig. 4.3; a full exemplar dataset is included in appendix C.2), demonstrating photoavoidance behaviour as expected, although they were observed to extend pseudopodia into illuminated regions in almost every image. Plasmodial morphology in these experiments was diminutive and the colonised oat flakes were typically only linked by a single plasmodial tube. After several days, the plasmodium was similar in appearance to the mask shape's morphological skeleton. This similarity may only be labelled as an approximation, as the tube networks typically had multiple unwanted (parasitic) branches.

When considering the imperfection of this approximation, it must be noted that there is some variation in conventional computer-generated skeletons arising from the differences between the various algorithms available for the task; consider that each of the results shown in Fig. 4.1B contain parasitic elements which do not accurately represent the 'C' shape. Accordingly, the majority of real-world applications of skeletonization algorithms (such as edge detection in machine vision applications) require additional processing (pruning) to remove these artefacts.

We may speculate that *P. polycephalum* assumes this morphology i.e. a minimal total edge length to represent the shape of a dataset, in such instances in order to maximise the efficiency of its nutrient harvesting networks when in the presence of copious but spatially-distributed nutrient sources; repellent optical stimuli may be used to confine the shape of the organism to the 'image' as represented by the opaque mask.



(a) (b)

(c) (d)

(e) (f)

FIGURE 4.3: Photographs to show results from single image experiments with a 'C'-shaped mask. (a,c,e) Propagation of 3 separate plasmodia after approximately 70 h. The organism tends to avoid areas outside of the mask but does occasionally stray outside of it. (b,d,f) Enhanced images highlighting areas of plasmodial growth; each image corresponds to its neighbour to the left. Elements of figure adapted from author's own work in Ref. [164].

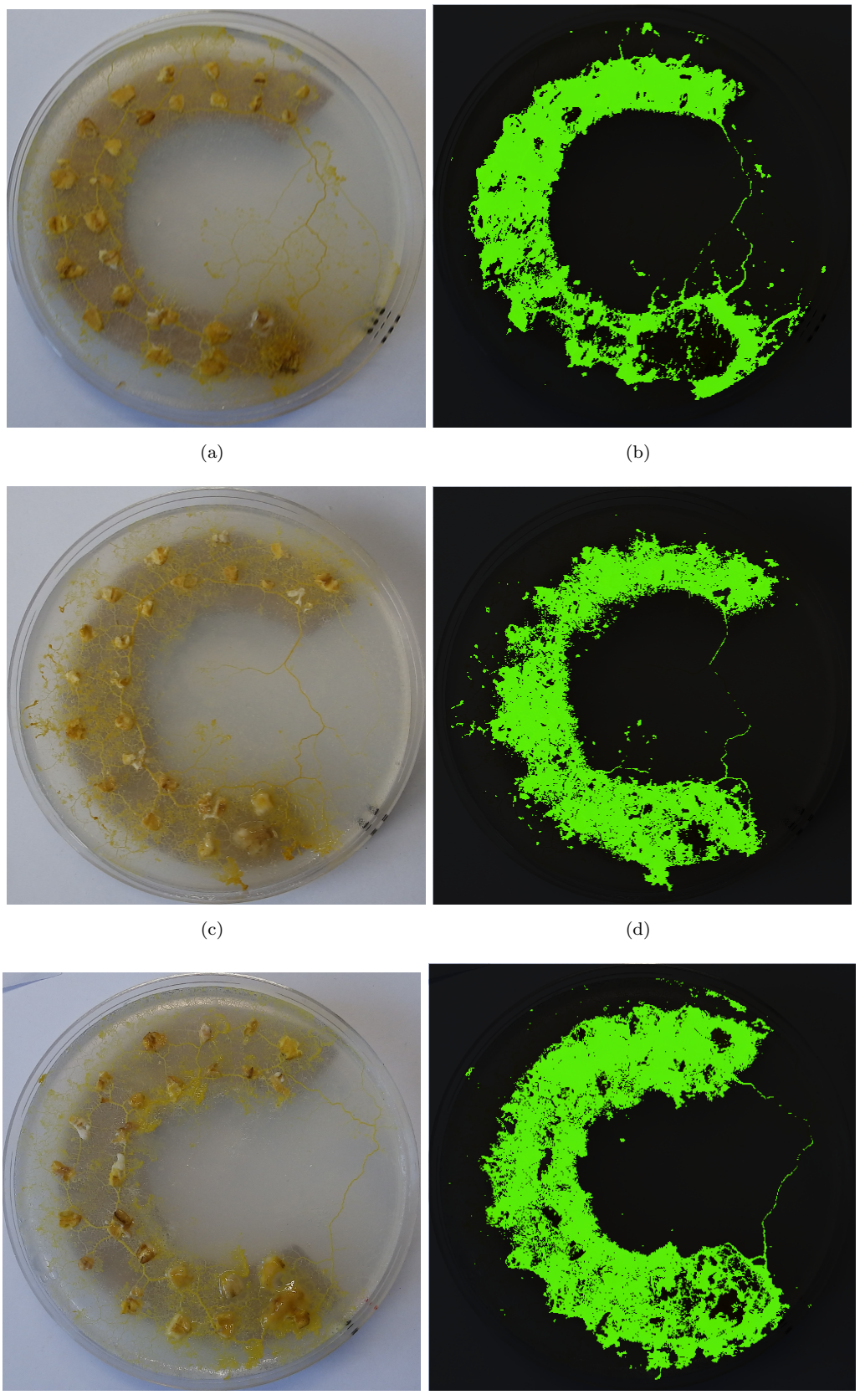
4.1.3.2 Double image experiments

The key finding of the double image experiments was that this treatment causes the *P. polycephalum* plasmodium to become almost entirely confined to the shapes represented by both chemoattractant distributions and optical fields, which also caused it to construct markedly different plasmodial tube network morphologies than those observed in single image experiments. As is demonstrated in Figs. 4.4 and 4.5 (a full exemplar dataset for each shape are included in section C.2), the organism attempts to traverse the layer of naked, light-irradiated agar in all experiments, presumably to link its tube network in a closed ring once it had initially colonised the shaded areas. In the 'C' shape example, the organism opts to migrate directly across the shape's concavity and constructs relatively faithful simulacra of the convex hull in doing so. In the 'H' shaped examples, the organism only migrates across one of the two concavities and so does not construct a true convex hull.

4.1.3.3 Enhanced light intensity experiments

These results pose the question as to why the organism was willing to approximate the convex hull of the 'C' shape but not the 'H'. The fact that the convex hull was essentially 'half' constructed in the latter would seem to imply that the plasmodium is tolerant to extending only a certain percentage of its body through such regions, presumably dependent on the comparative intensity of the light (i.e. strength of repulsion) and the organism's size and health status, such that the detrimental effects of light irradiation (dehydration, UV light-induced DNA damage) are offset by the benefits to the organism of having a more efficient nutrient harvesting/distribution network. The results gathered were insufficient to make an informed estimation of these quantities, however.

In order to evaluate this hypothesis, the 'C' shaped single and double image experiments were repeated using an additional source of illumination, a 20 W 180 cd UV strip light, which was utilised under the rationale that the organism is intensely phobic towards UV light [53]. The double image experiment detailed in Fig. 4.6 (data for single image not included), illustrates how the growth of the plasmodium in such scenarios is almost entirely confined to the area within the mask: initial growth patterns appear similar to the double image experiments but once the entire mask area had been colonised, the organism proceeded to traverse back along routes it has already colonised, rather than leaving a diminutive tube network. This morphology is not usually observed as *P. polycephalum* will tend to migrate away from areas it has previously colonised in order to distance its self from the metabolic by-products it excretes in its slime layer [43], hence this higher surface area morphology would appear to represent the organism's attempts

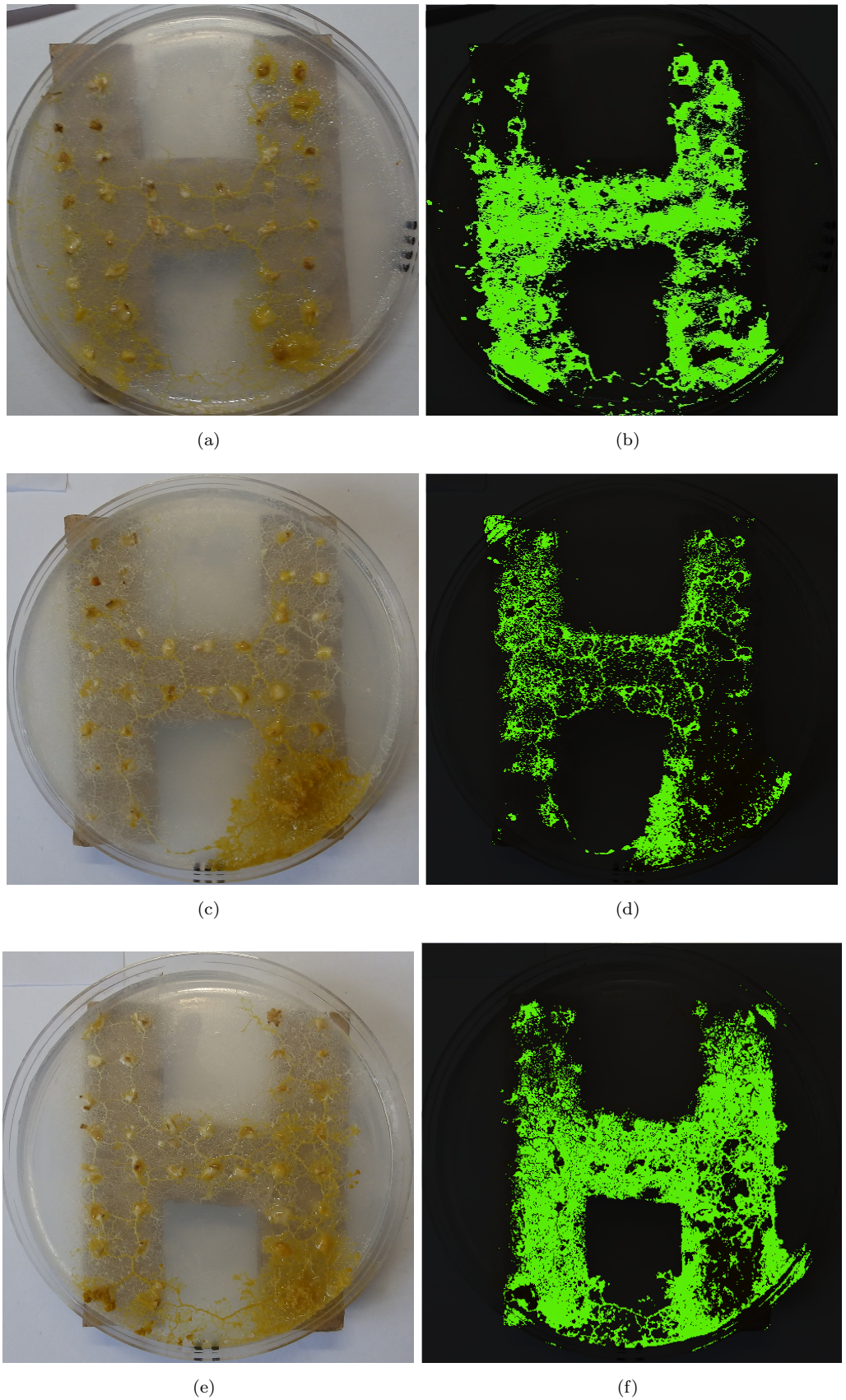


(a) (b)

(c) (d)

(e) (f)

FIGURE 4.4: Photographs to show results from double image experiments with a 'C'-shaped mask. (a,c,e) Propagation of 3 separate plasmodia after approximately 69 h. (b,d,f) Enhanced images highlighting areas of plasmodial growth; each image corresponds to its neighbour to the left. Elements of figure adapted from author's own work in Ref. [164].



(a) (b)

(c) (d)

(e) (f)

FIGURE 4.5: Photographs to show results from double image experiments with a 'H'-shaped mask. (a,c,e) Propagation of 3 separate plasmodia after approximately 65 h. (b,d,f) Enhanced images highlighting areas of plasmodial growth; each image corresponds to its neighbour to the left. Elements of figure adapted from author's own work in Ref. [164].

to utilise all available space excluding its own trails. In these experiments — which were also highly repeatable — the organism's morphology was an accurate representation of the mask image.

This demonstrates how modulation of the relative strengths of attractive and repulsive forces may be used to dictate the way in which the organism will represent the 'images' which are presented to it as sensory input. These results suggest that further experimentation into varying the intensity and colour of light used may prove fruitful in enhancing control over the SMMP.

4.1.4 Modelling Results and Discussion

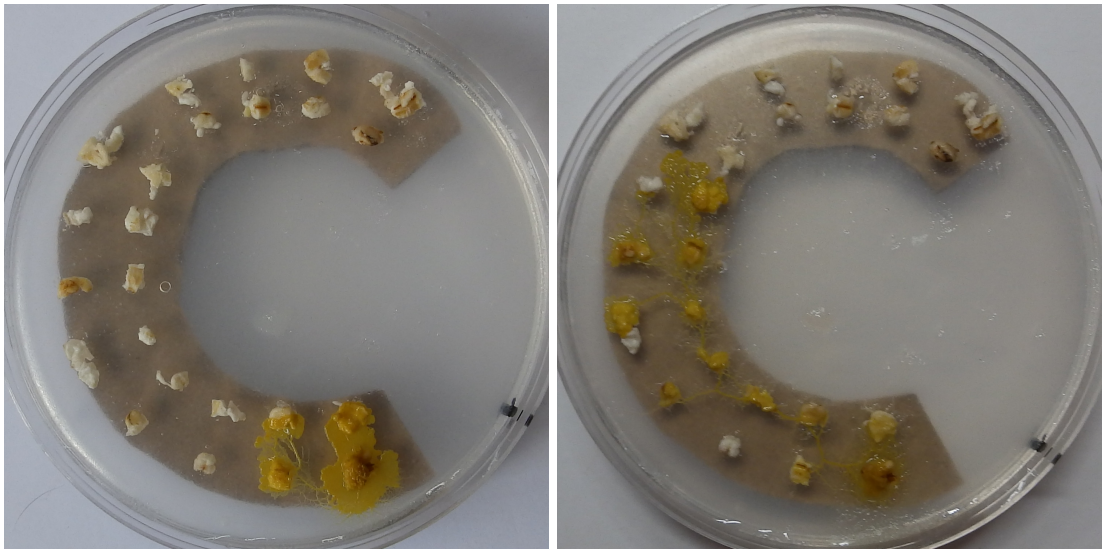
The multi-agent model was able to accurately replicate the organism's colonisation of unilluminated, nutrient-rich areas in simulacra of the double image experiments, in which the virtual plasmodium grew into an approximation of an interconnected concave hull (Fig. 4.7). Conversely, the entire environment was colonised in control simulations. The model was not able to reproduce the eventual formation of the convex hull of either shape, however. This highlights the discontinuity between real and virtual plasmodia, as the former exhibit a certain tolerance to spanning light-irradiated areas. Results for single image experiment simulations were very similar to those for double image experiments (data not included).

Illuminated areas were represented in the model by reducing the ability of constituent particles to sense attractant gradients; whilst this is not a faithful reproduction of the mechanisms involved (as was discussed in section 2.2.5, the activation of cytoplasmic photoreceptors generates a repulsive signal rather than damping attractive ones) and hence a feasible explanation for the source of discrepancy between real and simulated results, it should be noted that observations such as in Fig. 4.7 were more akin to the enhanced light intensity experiments, despite the model not replicating the eventual dilation of the real plasmodial networks.

4.1.5 Further Discussion

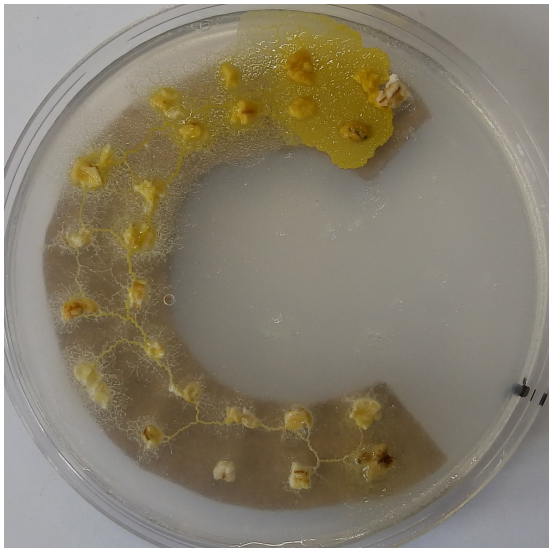
The principal findings of this investigation are twofold:

1. The *P. polycephalum* plasmodium is capable of perceiving 'images' represented by spatially-distributed sources of attractive and repellent stimuli — although of course the organism cannot be said to conceptualise this spatial information as an image in the sense that we would understand it — and will adapt its somatic

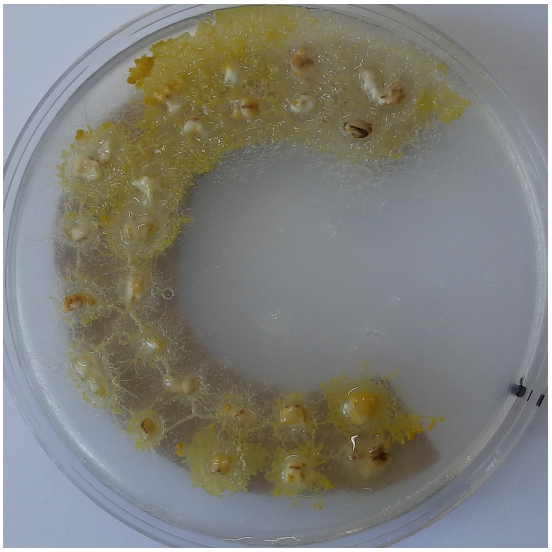


(a) 3h

(b) 10h

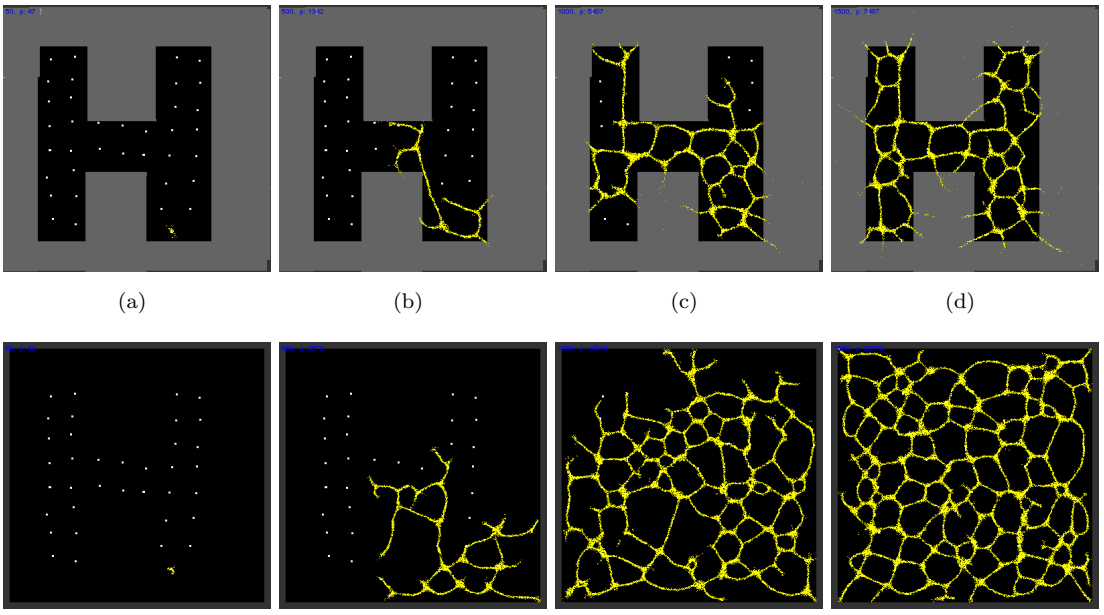


(c) 24h



(d) 51h

FIGURE 4.6: Double image 'C' shape experiment where an additional UV light source was used to augment the strength of optical repulsion. Although growth patterns are initially similar to original double image experiments, the organism is unable to traverse illuminated regions and hence proceeds to fully occupy the space under the mask, creating an accurate representation of its image in doing so.



(a) (b) (c) (d)

(e) (f) (g) (h)

FIGURE 4.7: Multi-agent model of double image 'H' experiment. (a–d) Growth of virtual plasmodium in a simulacrum of the laboratory experiment in Fig. 4.5. The resulting network approximates the shape of the mask and attractants but is too interconnected to be labelled as a hull. (e–h) Control experiment where no repellent was applied. The entire area becomes colonised. Adapted with permission from Ref. [164].

morphology into a representation of its surroundings. The organism can approximate the morphological skeleton and convex hull of a shape, as dictated by the comparative strengths of the attractive and repulsive forces employed; whilst the data presented does not provide strong evidence to indicate whether or not the plasmodium can approximate the concave hull of a shape, it is clear that it is able to represent the complete shape of a dataset when mediated by overwhelmingly repellent stimuli.

2. The laboratory experimental results presented are substantiated by and in-turn somewhat verify the applicability of Jones' multi-agent particle model of slime mould migratory dynamics. Several discrepancies between the real world and simulation exist, most notably the absence of convex hull approximations which are likely to result from a lack of fine-tuning of the parameters which represent repulsive fields.

This ability to represent shapes mediated by environmental stimuli expands the scope of the SMMP beyond simple subdivision of space applications to encompass the realm of image processing. Beyond this, however, (as we concede that the uses of the specific SMMP devices presented here are somewhat trivial), we have observed through these exceedingly simple, crude experiments how an unadulterated plasmodium is able

to continuously and dynamically translate environmental sensory data into a coherent behaviour pattern that represents the sum of a complex network optimization problem. This is achieved through cooperative coordination of every part of its body in the absence of a centralised control system: in effect therefore, the organism's many appendages should be thought of as computational resources, as its momentary network morphology will determine how it reacts to consequent sources of input.

As with the heterotic devices presented in Chapter 3, however, the practical uses of the SMMP are somewhat confined; we have, however, demonstrated here that the SMMP has more functions than previously described and that the use of multiple formats of simultaneous input is a viable method towards this i.e. polyfunctionality is a product of polymorphism.

This behaviour is perhaps easily dismissed as optimised foraging behaviour from a biological perspective, but it is complementary to recent advances in artificial intelligence which suggest that an entity with a compliant body that exhibits complex non-linear dynamics possesses significantly more computational resources with which to achieve greater heights of apparently 'intelligent' behaviour [141, 235]. This concept, known as morphological computation, relies on exploiting the properties of the materials of which an entity is composed (typically in the context of robotics) in order to 'outsource' a certain percentage of computing work to its morphology[4]. But robots — and even other biological entities — cannot usually completely re-structure themselves to suit a specific task, implying that slime mould is a far more adaptable platform for instances where morphological adaptation is desirable. Thus, we conceive that morphological processing of environmental sources of input represent a huge area of potential applications for the SMMP, as the results presented here demonstrate a unique linkage between entity and environment; that we have identified the extent to which this behaviour is reconfigurable but essentially programmable is merely the first step towards this goal.

To conclude this section, we present the following areas for further study.

1. Further experimentation with different shapes and stimulant intensities in order to achieve fine-tuning of the organism's abilities to implement morphological operations.

2. Implementation of minor adaptations to the multi-agent model.

3. Deeper investigation into how SMMP morphology reciprocally reflects details about the organism's environment.

[4]This concept is explored further in Chapter 5.

4.2 On Coupled Oscillator Dynamics and Incident Behaviour Patterns: Emergence of Wave Packets, Global Streaming Clock Frequencies and Anticipation of Periodic Stimuli

4.2.1 Introduction

Optical interpretation — whether by eye or machine vision — of slime mould migration patterns has formed the sole basis of output interpretation in SMMP prototypes produced to date. Whilst this computationalist view of slime mould foraging behaviour has found practical applications mainly in the field of image processing, this is nevertheless a narrow scope for such a substrate. Furthermore, in purely practical terms their operation is very slow and requires significant amounts of user input in their setup and interpretation of results. The purpose of the investigation described here was to extend the range of uses for the SMMP beyond image processing by exploiting a desirable emergent property of the organism — its ability to display apparently anticipatory responses to periodic stimuli — as a computational resource.

4.2.1.1 Anticipatory behaviour: definitions

The foundations of modern thought on the study of anticipation were laid by Rosen in 1985 [250], who argued[5] that biological organisms capable of this behaviour must have the means to:

1. Perceive sensory input pertaining to the state of the immediate environment.
2. Encode sensory information into a format the organism can unambiguously interpret, such that it has momentary awareness of its environment.
3. Store information pertaining to previous environmental states in some form of memory.
4. Construct an internal simulation of likely future events based on current sensory information and memorised past states, such that the organism can change its momentary behaviour, i.e. in anticipation.

[5] Rosen's work concerned a rigorous discourse pertaining to the philosophical, mathematical and biomolecular bases of anticipatory behaviour, that he compiled in his efforts to develop his field (systems biology) as a means of describing complex biological phenomena which contemporary physics had, in his view, failed to account for. He concluded that anticipation in a biological system is essential for adaptation and learning.

These statements all make reference to concepts that are readily transferable to UC and indeed, Rosen's work has been subsequently built upon by Dubois [99, 101] who abstracted the concept to apply to any entity, including artificial intelligences, by laying down the mathematical foundations for how algorithms should be designed in order to replicate Rosen's anticipatory systems. Dubois draws distinction between two types of anticipatory system, those which construct the future simulation internally ('strong' anticipation) and those which construct (or have the simulation constructed for them) externally ('weak' anticipation), that conceptually distinguishes between autonomous and non-autonomous systems, respectively. This is the basis of 'Dubois' conjecture', which states that autonomy implies that supplementary information is generated by and consequently added to a system in the process of processing external information. Harnessing this ability for UC purposes is highly desirable as it forms the basis of creating complex, adaptable systems that share emergent features usually assigned to higher forms of life.

Translating these terms into a more biological context, Collier [76] (in his works on the epistemological foundations of classifying anticipatory behaviour) argues that all living systems can be said to exhibit the 'strong' variety of anticipation because their actions are autonomous. Collier's hypothesis is dependent on defining organisms as having a teleological purpose: survival, regulated by autonomous (genetic) self-preservation responses. This is of course a contentious issue as the Darwinist interpretation of survival is that any 'purpose' an organism may display is only apparent, i.e. teleonomical. It is beyond the remit of this investigation to explore this concept further, but suffice it to say that we regard it as irrelevant whether an organism exhibits true or apparent purpose in its ability to project internal simulations of past, present and future events as well as make decisions pertaining to momentary behaviour based on these: this is autonomous (i.e. self-regulating) in origin and will presumably confer some form of survival advantage whether it can be said to be 'purposeful' or not. Thus, as slime mould has been described as exhibiting anticipatory behaviour, we may hypothesise that the processes which underlie it are algorithmic in nature and therefore a computing resource that can be exploited for the generation of UC devices with a wide range of novel applications.

The anticipatory behaviour of slime mould has only been reported in one experimental study by Saigusa *et al.* [254] — which demonstrated that the organism will slow its crawling speed in apparent anticipation of a rapid, prolonged (60 min) combined temperature and humidity drop that had been previously applied periodically at least three times — and though several hypotheses have been made pertaining to how the organism is capable of such behaviour, the phenomenon is still poorly understood.

In this investigation, we initially present a literature review on the bases of anticipatory behaviour in slime mould followed by laboratory experimental studies in which we successfully induce anticipatory behaviour in the *P. polycephalum* plasmodium via a well-characterised mechanism — photoavoidance. We proceed to extrapolate the biomolecular basis for slime mould anticipation and conclude by discussing the computing applications of anticipatory SMMPs.

4.2.1.2 Anticipatory behaviour as a function of coupled oscillator dynamics

The current scientific consensus is that anticipatory behaviour arises in biological, chemical and physical systems as a result of non-linear interactions between coupled chaotic oscillators which bring them into or out of synchrony. It is now a well-observed phenomenon that coupled dissipative chaotic systems may bring themselves into some degree of synchrony, either spontaneously or gradually, as a function of the method of linkage between them. This is especially true of biological systems: to consider the topical example of *P. polycephalum's* shuttle streaming oscillator, although the rhythmic contraction and dilation of the organism is harmonic, the biomolecular processes which drive it — biochemical signal transduction pathways, enzymatic reactions etc. — are distinctly chaotic (see section 4.2.1.3), thus highlighting the extraordinary power of biological material for 'taming' chaos [227].

Several varieties of oscillator synchronisation have been identified including the emergence of complete synchrony (usually in identical oscillators) [289], phase-only synchronisation [252] and lag and anticipatory synchronisation [251, 318]. This latter category refers to a phenomenon observed when one or more oscillators slaved to a master oscillator will adjust their motion in a manner that appears to pre-empt perturbations in the master when the system has been sufficiently entrained through external input so as to attain some stability. The concept of entraining a master oscillator through external input is a proposed mechanism underlying the emergent behaviours we label as anticipation in autonomous systems, and whilst differences in opinion exist as to the exact nature of the phenomenon, several biological systems have been experimentally demonstrated to exhibit anticipatory synchronisation of underlying oscillators when exposed to some form of periodic exogenous stimuli. Some examples of such systems include:

Simulation Coupled Rössler oscillators [59], Lorenz systems [242] various CA rules that generate periodic fractal patterns [100], simulated neuron cultures [72, 242, 318], Lyapunov-Krasovskii functional (linear time delay) systems [224].

Physical Coupled bistable circuits, Rössler oscillators [238], Hindmarsh-Rose neuron circuits [242].

Chemical Reactant profiles in the Belousov-Zhabotinsky reaction [221].

Biological Neural cell culture bioelectrical activity [241], competing nerve relay oscillation controlling circadian rhythms in *Drosophila* flies [286] and food anticipation in mammals [273].

Accordingly, the hypotheses advanced to date concerning the phenomena which underlie slime mould anticipatory behaviour patterns make reference to the concept of anticipatory synchronisation in oscillating intracellular systems. In the seminal article on the topic [254], the authors presented a non-specific dynamical systems model which mathematically demonstrated that variables in a generalised oscillator equation will eventually synchronise with periodic external input. This was later built upon by Pershin *et al.* [232] who suggested that the dynamical history of the organism's external input was 'stored' as the momentary pressure differential exerted on its thixotropic intracellular environment with the changes in environmental temperature and humidity used as input: this in turn, they claimed, altered the flow rate of shuttle streaming and hence changed its crawling speed. They concluded by likening this phenomenon to the alterations in electrical resistance a memristor exhibits in response to various magnitudes of electrical input. Although this theory was well accepted and received press attention over a period of years — likely due to the novelty of memristors since their first experimental demonstration in 2008 and the discovery by Gale *et al.* in 2013 [126] that slime moulds are electrically memristive — it does not consider any of the biomolecular processes which drive the maintenance of intraplasmodial pressure or indeed the ramifications of this on any other specific processes.

Aside from the incompleteness of the 'memristor analogy', it also implies that the hypothetical behaviour of slime mould is not autonomous and is mediated entirely by exogenous forces and hence, only weakly anticipatory as per Dubois' classification. Furthermore, it poses several unanswered questions, such as whether the organism can display this behaviour in response to input which does not alter its intracellular pressure and how non-immediate memory is maintained when the intracellular pressure is constantly fluctuating in response to ectoplasmic actomyosin contraction. This presents the necessity of approaching the characterisation of slime mould anticipatory behaviour from a biological perspective as it is only through the identification of underlying processes that such phenomena can be manipulated effectively. In acknowledgement of this, the following section contains a brief literature review of the nature of the organism's oscillating cellular processes which indicates the likely biological basis of slime mould anticipation.

4.2.1.3 A review of slime mould oscillators

As was mentioned in section 2.2, the *P. polycephalum* plasmodium physically oscillates via the contraction of radially, longitudinally and spirally-orientated ectoplasmic muscle proteins (actomyosin complexes) [44]: as a result of these contractions its cytoplasm is propelled rhythmically back and forth along its anteroposterior axis which provides motive force and distributes the contents of the cytoplasm throughout the organism. The period of this process is 60–120 s and can be influenced by altering the favourability of its environment. Net forward migration is achieved thanks to the thixotropic nature of the intracellular environment: asynchronous contraction generates a pressure differential which precipitates anterior solation and posterior gelation. This process may be considered as being a biomechanical phenomenon driven by the physical contraction of actomyosin proteins, hence intraplasmodial pressure peaks in phase with the tension generated by protein contraction as well as cytoplasm propulsion velocity.

An interesting phenomenon related to the biomechanical oscillator is the emergence of a global (organism-wide) streaming 'clock' frequency. A scant few pieces of historical literature report that the streaming frequency of individual plasmodial tubes will, when disturbed by some form of stimulus, revert to the global frequency after a short period of time has elapsed. Furthermore, a plasmodial tube transplanted onto a different organism will rapidly (in under an hour) synchronise its streaming frequency with that of the new plasmodium [332, 343]. Although little reference to this phenomenon is made in literature other than these two articles, Dietrich notes in his retrospect on slime mould biology research that its underlying mechanisms were sought after in the 1970's [94]. For our purposes, the issue as to whether or not *P. polycephalum* has a master oscillator to which other oscillating intracellular systems are slaved is a key research question.

Plasmodial membrane potential oscillates in synchrony with the biomechanical oscillator, although the two systems may occasionally be observed to be slightly out of phase [168]. These two systems are not directly coupled to each other, however, as experimental studies have demonstrated that bioelectrical phenomena do not precipitate muscular contraction [331] and equally membrane potential continues to oscillate when protein contraction has been chemically restrained [177, 308]. The key and likely sole endogenous determinant of plasmodial membrane potential[6] is the intracelluar concentration of free hydrogen ions ($[H^+]$, where brackets denote 'intracelluar concentration of') [114, 156],

[6]Note that plasmodia not at rest, i.e. stimulated, will display membrane potential modulation in response to various sources of input [225].

rather than calcium ions ($[Ca^{2+}]$) as several studies have stated [33, 213, 274][7]. $[H^{+}]$ levels oscillate in resting plasmodia due to their being produced as a by-product of the biochemical reactions that produce energy in the form of ATP, which are collectively known as oxidative phosphorylation: this adequately explains how membrane potential continues to oscillate in the absence of actomyosin contraction as energy production must necessarily continue unabated within the organism at all times. Accordingly, [ATP] oscillates in synchrony with tension production [345]. Thus, the organism's metabolic oscillator is the master oscillator to which the biomechanial and bioelectrical systems are slaved.

Despite $[Ca^{2+}]$ not being a determinant of membrane potential, a great many studies have demonstrated — through the use of chelating agents to sequester intracellular calcium [199], remove the endoplasm and replace it with artificial media [309] and removing calcium sources in the organism's foodstock [332] — that the organism requires calcium in order to undergo muscular contraction and that the cytoplasmic concentration of free calcium oscillates over time in approximate antiphase of the biomechanical oscillator [346]. This suggests that, contrary to its expected functions, calcium has a pro-relaxation effect on actomyosin contraction: a significant number of both *in vitro* and *in vivo* studies have elucidated the mechanisms underlying this behaviour, which seem to stem from calcium-based activation of factors that suppress contraction such as myosin II and fragmin [167, 170, 172, 179, 182, 344, 346]. This is further supported by a mathematical model of the slime mould calcium and energy supply oscillators by Smith and Saldana [278], which accurately replicates the behaviour of coupled calcium, biomechanical and bioelectrical oscillating systems. Rhythmic changes in free calcium concentration are coordinated by its sequestration in intracellular vesicles which is precipitated by increases in [ATP], although the relative concentrations of vesicle-bound to free calcium over time are poorly characterised [44]. Hence, a supplementary system of biochemical oscillators are also slaved to the organism's energy supply system.

The oscillators detailed in this section comprise the major oscillating systems within the organism: a limited number of other slime mould life processes are known to oscillate — e.g. cyclic adenosine monophosphate (cAMP) concentration (a signal transduction protein), the phase of which precedes calcium oscillations by about 60^{o} [312] and the kinase phosphorylation fraction (percentage of proteins 'activated' by kinase enzymes in certain biochemical pathways) protein load, which is in phase with tension production

[7]This misconception originated from a combination of the known effects of calcium on precipitating muscle contraction in human striated muscle and a 1979 study by Meyer and Stockem [213], who studied changes in fluorescence intensity over time in plasmodia treated with a fluorescent calcium dye and, on observing direct correlation between fluorescence and biomechanical oscillation, claimed that the two were linked. Their results were not calibrated to compensate for changes in plasmodial thickness (i.e. fluorimetric imaging rather than ratiometric), however, despite previous literature on the topic warning against this eventuality [253], and consequently their results were called into question [44, 333].

— but these are considered to be by-products of the other reactions occurring. Furthermore, genetic factors such as the cell cycle also oscillate but as these are very slow (hours to days in duration), open-loop systems, they cannot be considered to underlie the rapid, reflexive anticipatory responses under investigation. Crucially and somewhat surprisingly, the concentrations of several compounds essential to the functioning of the various major oscillating systems described above do not oscillate, such as the adenine nucleotides which are used in the synthesis of ATP and various allosteric enzymes involved in the biosynthesis of other precursors to the energy supply system [312].

In summary, we have characterised various oscillating slime mould life processes as biomechanical, bioelectrical and biochemical systems, all of which are slaved to an oscillating master energy (ATP)-generating pathway. The relative phases of various consituent oscillators are shown in Fig. 4.8.

4.2.1.4 Hypothesis

We have seen that coupled chaotic oscillators display the following behaviours:

1. Phase synchronisation of multiple slave oscillators to that of a master oscillator when the system is in an equilibrium state.
2. Phase shifts in slave oscillators sympathetic to entrainment by perturbations of the rhythm established by the master oscillator, whose motions alter in such a way that they appear to anticipate similar future perturbations.

We have identified that of all of the intracellular oscillating systems within *P. polycephalum*, several subsystems which control key cell functions are slaved to the master energy supply oscillator: hence, we can speculate that this clock frequency may be the origin of the underlying streaming clock frequency (i.e. when the system is at rest) as well as anticipatory behaviour exhibited by slaved subsystems. This poses the vital question as to how the master oscillator becomes perturbed such that its dynamics are sufficiently altered as to elicit the knock-on effects on its slaves.

The following investigation was based on the hypothesis that adverse stimulation of the organism causes modulation of its intracellular biochemical environment through altering the availability of compounds which influence the energy supply system, such as allosteric molecules (this in effect provides a basis for explaining the conversion of polymorphic input streams into a coherent output). This may feasibly bias the master oscillator. To this end, the *P. polycephalum* plasmodium was periodically subjected to adverse stimulation using a method whose biological effects are better-characterised.

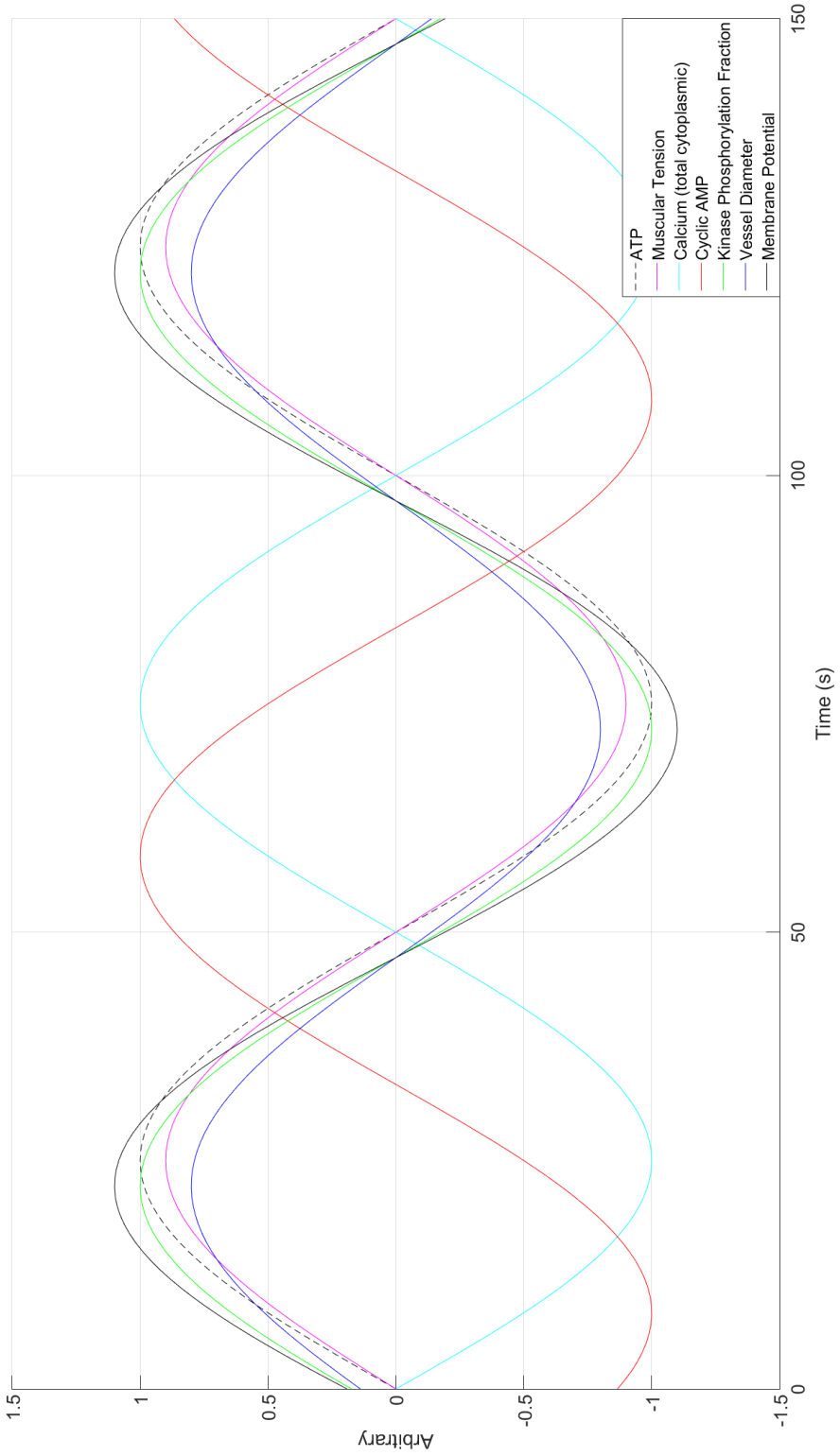


FIGURE 4.8: Graph to show relative phases of *P. polycephalum's* intracellular oscillators, derived from experimental studies. Membrane potential, vessel diameter and phosphorylation fraction are in phase, as are total cytoplasmic ATP and muscular tension; the former leads the latter by approximately 10^o. Free calcium concentrations are 180^o out of phase with ATP/tension and cAMP lags 60^o behind calcium. X-axis values and wave characteristics are arbitrary as graph shows only relative phases. Figure adapted from author's own work in Ref. [208].

Optical stimulation through irradiation with UV light was chosen as the adverse stimulus as the organism is known to possess several UV light-sensitive cytoplasmic photoreceptor proteins; it readily exhibits photoavoidance (implying up-regulation of the biomechanical oscillator in order to achieve net migration) to low-intensity UV light but will undergo spontaneous sporulation if subjected to higher doses [282]. Hence, the effects of such treatment are visible as an alteration in the organism's streaming patterns: this was observed through low-magnification examination of plasmodial tube contraction and dilation as well as higher-magnification observation of the organism's actin network, which was performed in order to concretely ascertain whether the treatments administered were having direct effects on normal patterns of muscular contraction.

4.2.2 Methods

4.2.2.1 Slime mould cultivation

Stock cultures of *P. polycephalum* were cultivated as described in section 3.1.2.1.

4.2.2.2 Visualisation of actin

Plasmodial homogenate were inoculated onto 0.5 ml hemispheres of NNA overlying large glass microscope coverslips. Another NNA hemisphere was situated approximately 10 mm away and was loaded with a chemoattractant (oat flake) to encourage the organism to migrate between the two, leaving a small plasmodial network over the naked glass. This apparatus will henceforth be referred to as the standard slime mould microscopy environment (SSMME). The SSMME was then placed in a sealed plastic Petri dish and was left to propagate in the absence of light for 2–3 days.

Actin was stained by microinjecting a large plasmodial tube linking the agar hemispheres with a solution containing 100 nM of a fluorescent G-actin analogue (SiR-actin, Spirochrome, Switzerland) in deionised water, via a CellTarm microinjection system (Eppendorf, Germany) with a hollow glass needle, tip size < 30 μm. Approximately 750–1000 nl of staining solution was delivered. After 1 hour had elapsed, the organism was transferred to a Ultraview ERS FRET-H confocal microscope (Perkin Elmer, USA) for visualisation. The fluorophore was excited with a 568 nm laser. Imaging data was post-processed (colour assignment, contrast enhancement) with the Volocity software package (Improvision, USA).

4.2.2.3 Entrainment experiments

Plasmodial homogenate was transferred to plastic Petri dishes containing a layer of 0.5 mm NNA. The NNA layer was prepared with ultra-pure water and care was taken not to introduce bubbles whilst pouring in order to maximise its light transmittance. Several oat flakes were scattered about the periphery of each dish before they were left in the dark for several days to encourage the development of complex, robust networks of plasmodial tubes. Dishes were then transferred to an inverted light microscope, the stage of which was covered by an opaque box which prevented light contamination from external sources.

Large plasmodial tubes of diameters exceeding 300 μm were isolated and visualised under a 10× objective lens using a constant low-level 100 W halogen power supply supplying approximately 125 lx (1.8×10^3 erg/mm^2). The plasmodia under investigation were allowed 15 minutes to equilibrate to the alteration in light level and any heat generated by the illumination, which was found to be an adequate timeframe in scoping experiments to allow heat changes to stabilise.

The organism was periodically stimulated by exposing the area being observed to the microscope's 100 W mercury arc fluorescence lamp, which was outputting 165 lx (2.4×10^3 erg/mm^2) through a 340 nm (UV-A) filter. The halogen lamp remained on during all these periods. Patterns of stimulation were as follows:

$$R_1 \rightarrow S_1 \rightarrow R_2 \rightarrow S_2 \rightarrow R_3 \rightarrow S_3 \rightarrow R_4 \rightarrow A \tag{4.1}$$

where:

- R_x phases correspond to 'rest' periods where the organism was not stimulated with UV light. These phases were of 900 s duration.
- S_x phases were 120 s periods where the organism was exposed to UV light.
- The final A phase which corresponds to the periodicity and duration of S phases but where no stimulation with UV light was provided and the streaming patterns of the organism were assessed for the incidence of anticipatory behaviour.

Control experiments were performed by exposing a tube to continuous illumination with the same halogen light source with no stimulus periods. The decision to perform three stimulation periods was made based on scoping experiments which indicated that two were insufficient to provoke a response in the third S window when no stimulation was

provided. These scoping experiments also informed the decision of phase durations as it was discovered that 900 s was sufficient to allow the organism to return to normal streaming patterns and 120 s of UV exposure did not cause rapid retraction of the plasmodial tube under investigation.

All experiments were filmed with a SC30 digital microscope camera (Olympus, USA). The diameter of the plasmodial tube was measured manually every 500 frames (approximately 14.5 s, totalling some 280 measurements per experiment) using CellSens software (Olympus, USA). Measurements were made perpendicularly to the vessel originating from the same X,Y coordinate. Measurement data were analysed with Matlab 2015a (Mathworks, USA) using Fast Fourier Transform, periodogram and basic statistical functions. All experiments were performed in triplicate.

Stimulation effects were investigated by splitting resulting power spectra into separate phases and comparing the frequency of oscillation in each to control values. Values were compared to and normalised against the 'baseline' (clock) streaming frequency, which was calculated as the R_1 phase; results were presented as the difference in streaming frequency between R_4 and A phase with and without adjustment (deviation from the baseline frequency, 'drift'): the rationale behind this was to reduce the effects of drift from the clock frequency which may have occurred for reasons other than the treatments administered. Only dominant streaming frequencies were measured as the organism is known to exhibit wave packet behaviour.

4.2.3 Results

4.2.3.1 Imaging

Actin was found to exist in dense, highly interconnected networks about the cell's ectoplasm. Irradiation of a plasmodial tube with the 568 nm laser used to excite the actin probe was found to cause rapid (within several seconds as opposed to the standard 1–2 min cycle) contraction of the vessel, as is demonstrated in Fig. 4.9. Contraction magnitude was in a similar order to normal contraction patterns.

4.2.3.2 Entrainment experiments

Full datasets for the following experiments are included in appendix C.3.

Control

Control plasmodia (Fig. 4.10A) exhibited periodic oscillations in vessel diameter as anticipated. The frequency of oscillation in these plasmodia ranged from 0.008–0.013 Hz

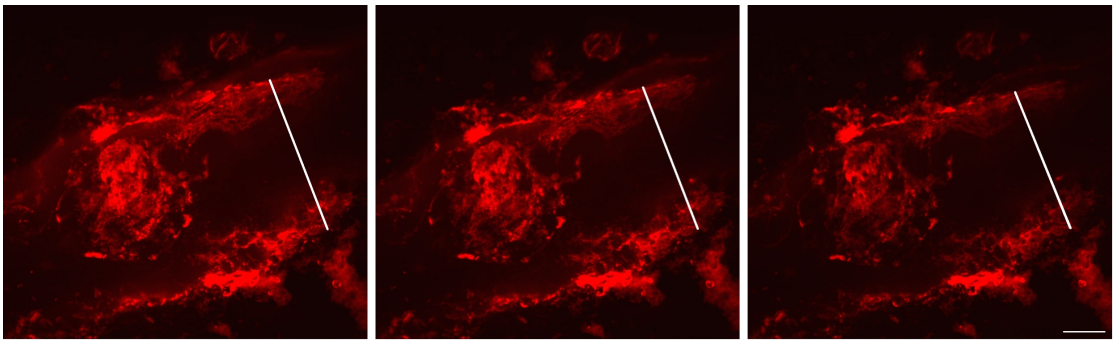


FIGURE 4.9: Sequential confocal micrographs showing the actin network (red) about a small plasmodial tube reducing in diameter over time (1833→1731→1678 μm) in response to irradiation with an intense 568 nm source. Time steps approx. 1 s. (a–c) Scale bar 250 μm.

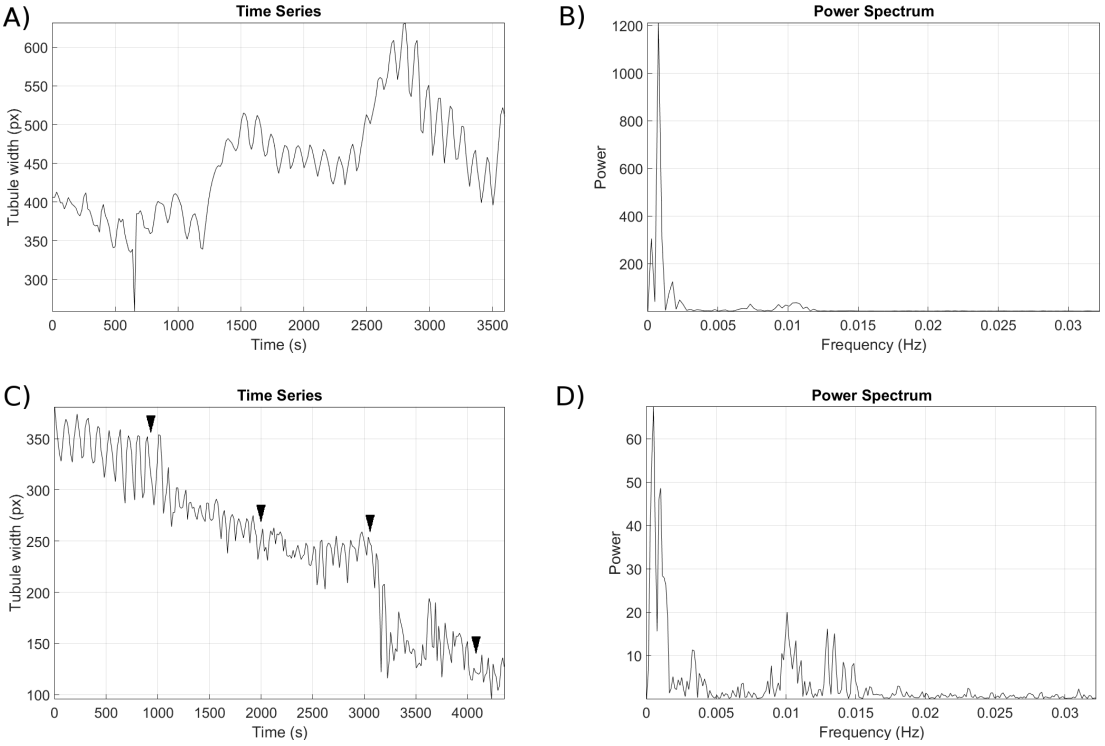


FIGURE 4.10: Graphs to show time series and power spectra for exemplar control and experimental datasets. (a–b) Control. Wave packets are visible. (c–d) Experimental. Phases are indicated in time series with arrows.

(mean 0.009 Hz). Wave packets of approximately 0.001 Hz were observed in all controls which were usually demarcated by characterstic 'jumps' in time series graphs. A general upward trend in tubule diameter was also observed in all experiments.

Experimental

Conversely, experimental plasmodia displayed a general downward trend in vessel diameter and tended to exhibit progressively chaotic patterns in amplitude and period of oscillation (Fig. 4.10B). Wave packets were not observed. Time series and power spectra

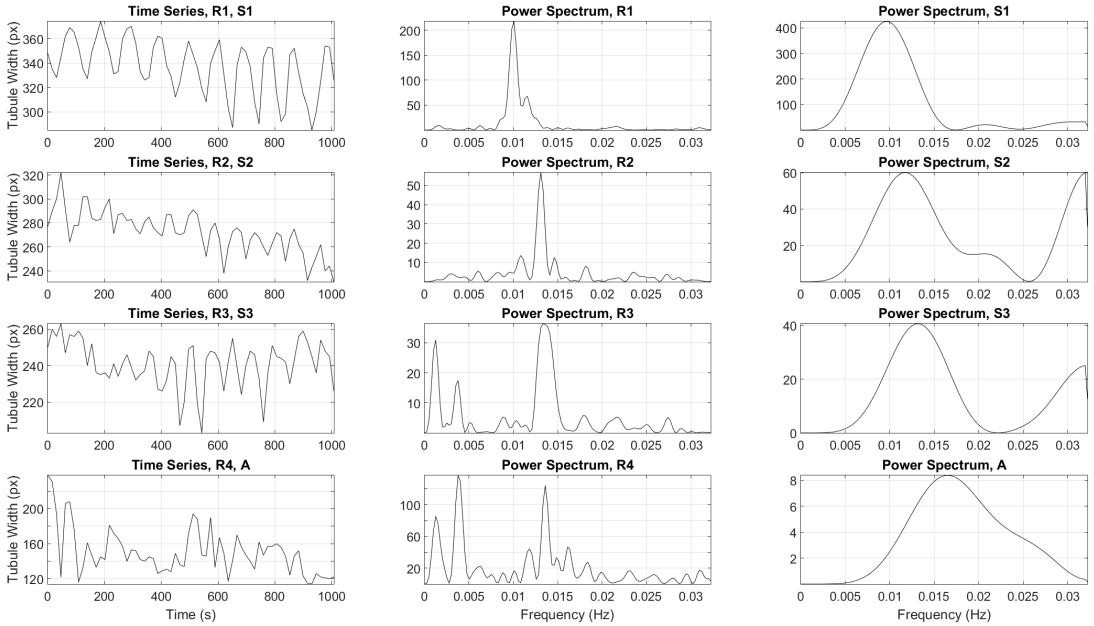


FIGURE 4.11: Graphs showing time series and power spectra for each phase of an exemplar dataset. Time series show both R and corresponding S/A phase. Dominant streaming frequencies gradually increase in both R and S phases, but reduce in power. A substantial, apparently anticipatory, frequency increase is evident during the A phase.

R_1 (BLF)	S_1	R_2	S_2	R_3	S_3	R_4	A	ΔAR_4 (%)
0.013	0.012	0.012	0.009	0.013	0.013	0.012	0.017	141.67

TABLE 4.1: Table to show streaming frequencies from exemplar dataset in Fig. 4.11. All values in Hertz except for ΔAR_4, which shows the percentage increase in streaming frequency between A and R_4 phases. BLF: baseline frequency.

		ΔfA (%)	$\Delta fA-$ BFD (%)
Control	$\bar{x}_c$	106.11	7.87
	σ	5.36	6.85
Treatment	$\bar{x}_t$	132.14	33.93
	σ	10.17	4.26
Difference	$\bar{x}_t - \bar{x}_c$	26.03	26.06

TABLE 4.2: Collated data to show the mean ($\bar{x}$) and standard deviation (σ) of A vs R_4 phase frequency changes (ΔfA), with and without baseline frequency drift (BFD) adjustment.

from an exemplar dataset split into separate phases are shown in Fig. 4.11B and the corresponding numerical data is shown in Table 4.1. Generally, streaming frequency would increase during initial S phases but would always tend towards being equal to the R_3 phase frequency by the S_3 phase. R phase dominant frequency tended to be relatively constant. A phase dominant frequency substantially increased in all such experiments by a mean value of 26%, both with and without adjusting for baseline frequency drift.

4.2.4 Discussion

4.2.4.1 Imaging study

The *P. polycephalum* actin network was demonstrated to contract rapidly upon exposure to the laser used to excite the fluorescent actin probe. Although the light source used was not of an UV wavelength, these experiments confirm the direct coupling between light irradiation and plasmodial tube contraction via innervation of the mechanisms it uses for shuttle streaming, i.e the biomechanical oscillator. Note that sample heating was assumed to be minimal in these experiments as laser exposure was typically intermittent over a period of not more than 4 s.

4.2.4.2 Entrainment of anticipatory responses

The key finding of this study was that the *P. polycephalum* plasmodium exhibits increases in streaming frequency consistent with the concept of anticipation following entrainment with adverse periodic stimuli.

Although control plasmodia were observed to undergo small streaming frequency increases, likely as a result of constant low-intensity white light illumination, these were significantly smaller than the increases observed in experimental plasmodia. This demonstrates that the organism may effect proportional responses to optical stimuli intensity, which implies that its control system is closed-loop: this is of particular relevance as it reduces the possibility of the behaviours observed resulting from UV-induced DNA damage (the consequences of which may include altered gene expression, mitotic stage reversal and total DNA content reduction [92, 130]), as genetic control mechanisms are open-loop. The organism's tendency to increase streaming speed but attempt to match R phase streaming speed during S phases implies that both are compensatory measures designed to minimise the deleterious effects of harmful stimulation, likely through promoting gradual retreat from the area.

We may extrapolate from this data that plasmodial photoreceptors are coupled to the biomechanical oscillator. This allows us to hypothesise that upon entering a high-energy state following a receptor-photon interaction, the resulting intracellular signalling cascade's[8] end products influence the plasmodial energy supply system, presumably by up or down-regulating it. This, in effect, equates to biasing the oscillator's phase portrait which therefore has knock-on effects on each of its slaved systems.

[8] All photoreceptor proteins — including those present in plants, bacteria, protists and mammalian cells such as those in the eye — function by catalysing a highly amplifying signal cascade which typically involves either the cAMP-dependent (via activation with protein kinase A) or the diacyl glycerol/inositol triphosphate-dependent (via phosphatidylinositol biphosphate and protein kinase C) pathways [1, 279].

This explanation would appear to agree with the fact that the responses observed were relatively rapid in biological terms (minutes), as second messenger signalling cascades are known to initiate the recruitment of rapid-reaction biomolecules such as protein kinases and other allosteric molecules whose functions are to activate or suppress various intracellular systems [1].

This theory implies that the organism uses one or more rapid-reaction biomolecule varieties as a transient chemical 'memory' which either oscillates with its own exogenously-generated period or exists in non-oscillating quantities for a certain period of time; their presence continues to bias the organism's energy supply oscillator in either case. As mentioned before, some such compounds (e.g. protein kinase C) have been observed to oscillate within the *P. polycephalum* plasmodium whereas others have not [43, 171], but our hypothesis does not necessitate that these factors oscillate or otherwise. This description is consistent with Dubois' and Collier's definition of 'strong anticipation', i.e. a self-generated model of future events derived from past experience, is present within the organism. Such a definition is partially in agreement with the aforementioned models of slime mould anticipation in that it involves coupled oscillating systems, but disagrees with them in that the basis of slime mould memory is chemical, rather than physical. Furthermore, we have suggested a specific, well-characterised system by which this is achieved and have advanced empirical evidence to this effect.

Adjustment of streaming frequency results for baseline frequency drift increased the magnitude of treatment effects between R_4 and A phases, which indicates that the organism's underlying streaming clock frequency (representing the energy supply system) may alter over time in order to compensate for the effects of local adverse stimulation.

4.2.4.3 Computing with live systems via manipulation of constituent coupled oscillators

To adapt such a complex system into a functional unconventional computing system would be an arduous problem worthy of a separate program of study in itself to complete. Hence, in this subsection we discuss how these phenomena may be characterised in the language of computation and how this implies that the range of uses for the SMMP are far more diverse than originally thought, in efforts to guide future study on the topic.

When viewed as a discrete device, a plasmodial tube being entrained in this manner may be considered as a three-input AND gate, allowing for the discrepancy between data type input (light) and output (optical recognition of streaming speed), as the state of the device will only change such that the output is TRUE (by way of an example, this could equate to a 25–30% increase in streaming frequency) during the A phase if three

'1's (denoting the presence of an input during S phases) are provided. A UC device built on the exact techniques used in this study would be impractical and slow in comparison to conventional hardware, although its speed of operation (approximately an hour per logical operation) is acceptable in comparison to other biological logical gates previously proposed, such as those operating on principles of gene expression. These proposed devices, which will henceforth be referred to as anticipatory logical gates (ALGs), nevertheless demonstrate the organism's ability to encode information — transduction of light stimuli into a structured format which is then transmitted to the relevant effectors and temporarily stored — and to demonstrate an entirely programmable computation within the organism. We conceive that the ALG could be developed in the following two ways:

1. **Up-scaling**: If multiple precisely-aimed, low-intensity UV lasers were employed, several plasmodial tubes may be stimulated at once. If its underlying mechanisms were suitably elucidated, this might used as a method of influencing the plasmodial streaming clock frequency, which is likely to reflect the sum of inputs from the frequency of each tube.

2. **Automated output interpretation**: Changes in vessel diameter may be optically-recorded via a computer with various techniques such as colour recognition or light transmittance (spectrophotometry). This would allow for faster, more dynamic gate operation and would also facilitate up-scaling. As such a device would qualify as a SMHP rather than SMMP, the FPGA-based interface presented in section 3.2 is a feasible development platform for slime mould anticipatory systems.

We concede, however, that such devices would have a very limited range of practical uses. This further highlights that the SMMP is an analogue machine which is ill-suited to implementing digital logic.

The way in which we have described the *P. polycephalum* plasmodium's store of certain oscillator-biasing biomolecules is directly analogous to the concept of an integrator in an analogue computer, as they directly influence the 'signal generators' of the organism's various oscillators to a degree proportional to the sum of the 'calculations' performed during previous exposure to UV light. This perspective significantly increases the computing potential of hypothetical devices: for example, as our results indicate that slime mould responses to illumination are proportional, this presents the possibility for computing of arithmetical operations. To delineate, in 2013, Blakey [60] hypothesised that if a plasmodium could be entrained to 'remember' the period of stimulation then analysis of streaming patterns allows us to derive the period of oscillation when it is unknown: if

this work were expanded so that either a periodic function could be derived from analysis of streaming patterns, or if the organism could be entrained with a periodic function such that the period could be derived, slime mould could feasibly be used towards implementing a small-scale (i.e. allowing for only small numbers) non-quantum realisation of Shor's factorisation algorithm. This is an enthusing prospect as there exists no efficient general-purpose algorithm for integer factorisation for conventional architectures; Shor's algorithm, which has still not been successfully implemented for many numbers on the quantum architectures it was designed for, may theoretically complete its function in polynomial time [271, 315]. Such an application, whose implementation is certainly feasible according to our results, is a true embodiment of UC as it presents a novel use for computing devices. Furthermore, our results raise interesting questions as to the nature of memory and the computing process in other biological substrates such as the human brain, but such a debate is beyond the scope of this investigation.

We are by no means the first to suggest that live cells may function as analogue computers as, despite the fact that many of the more widely-known extant biological UC prototypes attempt to implement digital computation (e.g. the forerunner DNA computers as per the initial designs by Adleman [37, 69]). Encouraging progress has recently been made towards implementing analogue arithmetical circuits in the genetic transcription mechanisms of live cultured cells [85]. Although these analogue genetic circuits have been developed significantly further than the ALGs presented here and are as such far superior in terms of speed and accuracy, they have not been described as being able to exhibit anticipatory behaviour; this demonstrates the novelty of the devices presented here.

4.2.5 Conclusions

We have verified the effects of light irradiation on the *P. polycephalum* plasmodium and demonstrated its ability to effect a highly repeatable anticipatory response proportional to the dose of UV light administered. This mechanism presents huge potential for expanding the uses of the SMMP into regions where the full resources of the organism are utilised towards out-performing conventional hardware in certain aspects of their operation.

Our suggestions for further study include:

1. Simultaneous measurement of streaming phenomena in multiple tubes in order to assess whether the light/UV irradiation influences global streaming frequency.

2. Further work on determining the range and extent to which the organism's responses are proportional to stimulus intensity and duration would be a profitable route towards greater control over devices relying on the phenomenon of slime mould anticipation.

3. Investigation into how long the plasmodial 'memory' response is conserved after stimulation has ceased.

4. Work on entraining the organism with a periodic function assessing the feasibility of implementing Shor's factorisation algorithm with slime mould.

Chapter 5

Slime Mould Intracellular Computation

No extant *Physarum* machine prototypes have capitalised upon directly manipulating microscopic intracellular systems or interpreting output at the sub-meso-scale. This severely limits the range of ways we may interact with a *Physarum* machine in both input format and output detection, their range of applications and the applicability of findings therein to other biological substrates. As such, a great many of the substrate's desirable characteristics which are touted as justifications for the use of biological substrates in UC — parallelism, spatial propagation of information etc. — have not been fully exploited.

This chapter details investigations which comprise the initial work in the development of slime mould intracellular processors (SMIPs). Initially, we present our theoretical work expounding the plasmodial cytoskeleton as an intracellular network for information structuring, transmission and processing, which is substantiated by cellular automaton (CA) modelling. Following, we demonstrate through laboratory experiments how the organism's actin network may be practically utilised as a medium for implementing collision-based computing.

5.1 Computing with Intracellular Protein Networks: Theoretical Underpinnings

Let us briefly re-visit the justifications for research into slime mould intracellular computation from Chapter 2. An incredibly large number of ordered, highly-regulated micro and nanoscopic processes occur simultaneously within a cell in order to maintain life. This implies that although a live cell possesses a great many degrees of freedom, control systems exist such that chaos is avoided (this concept will be explored in detail in section 5.4); evolutionary biology indicates that these self-assembling biochemical systems balance order with variability, thus live systems appear to have a clear advantage over non-biological UC substrates such as chemical reactors. We envisage that intracellular reactions and other energetic events are viable computing resources in their own right due to their comparative small scale, the existence of endogenous control mechanisms, amorphism, energy efficiency and production of stable, measurable output (both micro-scale and on a cell behavioural level). But where in the milieu of the intracellular environment is one to start when designing biological UC devices, especially when they are not all fully-defined? This section justifies the selection of a specific intracellular system — the plasmodial cytoskeleton — upon which the experiments comprising the remainder of the Chapter are focussed.

As we have seen in the previous chapter, the macroscopic optical outputs of a SMMP require the operator to appreciate the microscopic processes occurring within the organism in order to properly exploit its computational resources. Such approaches employ a 'top-down' methodology in which the underlying processes that occur within the slime mould are treated as a black box. Conversely, intracellular computation necessitates a bottom-up perspective. The potential benefits of this approach are clear: aside from miniaturisation (implying higher information density, scalability etc.), micro-scale processes tend to occur much more quickly and allow for continuous tracking of the computing process. Conversely, the detriments of this approach include a more technically complex procedure to observe and manipulate the processes involved and a requirement for the systems being manipulated to be well-characterised.

Our investigations outlined in Chapter 4 highlighted the importance of information structuring to facilitate data transmission, storage and processing and provide programmable behaviour; furthermore, we recall from Chapter 2 that this concept is extremely important in contemporary artificial intelligence sub-fields such as robot design, as the structure of data streams within an entity and the coupling between them essentially dictates how it can interact with its environment [123, 141]. This concept is known as 'morphological computation', which we will briefly describe here.

Several authors have emphasised the opinion that the requirement for data structuring is necessary to enable logical ability, memory and learning in artificial intelligence constructs [123, 191, 192, 235]. This is because concepts such as these can only become a reality if they are embodied in a way that permits information flows into and out of the organism's physical body as it interacts with its environment: the means of interaction employed by both natural and artificial entities are typically mediated via sensorimotor information flows ('data streams'). Consider, for example, the complex interplay between sensory input and motor feedback required when a human lifts a glass of water to take drink from it: the person's arm and hand muscles work in synchrony with tension and touch receptors to manipulate the glass, which forms the basis of the 'environment' under scrutiny. The person's brain must make constant re-adjustments based on sensory data pertaining to the vessel's weight, orientation and relative position in order to accurately guide it to their mouth.

The essence of morphological computation lies within the acknowledgement that artificial constructs should be designed in such a way that a portion of the computing work required by it is 'outsourced' automatically and at no extra energy expense to the morphology (informational or physical) of the entity [234]. This is a bio-inspired design concept. Consider, for example, that the trans-membrane receptor proteins coating the *P. polycephalum* plasmodium are not sensors in the way that a robot might use them as they are entirely autonomous, activating independently when they interact with the appropriate stimulus: a conventional electronic sensor, on the other hand, needs to be sampled at a certain frequency by a central control unit as it cannot function independently. The former is clearly far more efficient than the latter: indeed, given that the systems which inspired this concept evolved naturally is proof of this. The concept of morphological computation has already led to great advances in the field of bio-inspired robotics and artificial intelligence [141, 234] and some authors have suggested that the theory may be reverse-engineered in attempts to describe the characteristics of emergent natural systems whose dynamics cannot be defined in terms of classical mathematics, such as brain function [84].

Thus we are free to speculate that there exists within the *P. polycephalum* plasmodium a physical medium through which sensorimotor data streams are free to flow and interact. Whilst we have previously spoken of incoming data streams being structured in a chemical format, we discount the possibility of the data stream being entirely amorphous as simple chemical diffusion would lead to entirely unguided transmission due to the shuttle streaming oscillator regularly distributing the contents of the cytoplasm throughout the organism. This implies the existence of a medium through which structured data may travel and presumably interact.

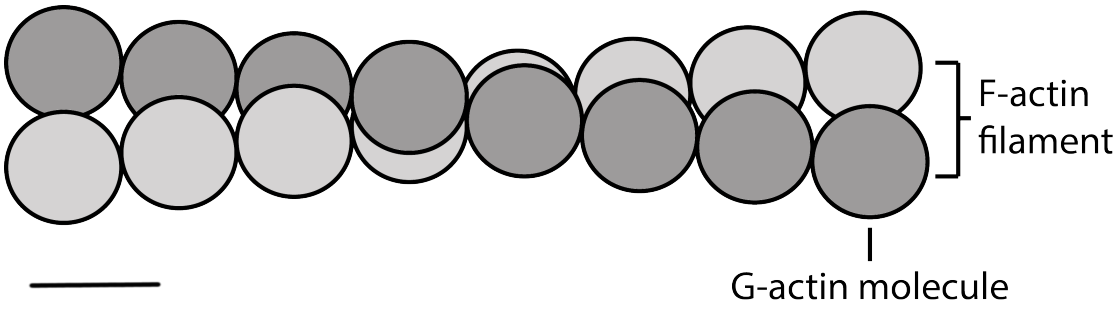


FIGURE 5.1: Schematic diagram of an actin microfilament. Scale bar = 5 nm, helix twist not to scale. Adapted from the author's own work in Ref. [202].

There does indeed exist a structure within the *P. polycephalum* plasmodium — and every other cell type, for that matter — which spans through the entirety of the cell and may transmit a variety of energetic events. This structure is known as the cytoskeleton, which is an extensive intracellular protein network formed from:

1. **Actin**. The most predominant protein in the eukaryotic cell, actin is a globular protein (g-actin) which assembles into long filamentous 5 nm wide double helices called microfilaments (f-actin) (Fig. 5.1) [78]. The functions of actin are known to include participation in cell signalling, transduction of mechanical force and participation in muscular contraction — and hence, cell motility — when complexed with myosin (actomyosin).

2. **Tubulin**. Also a ubiquitous cytoskeletal protein, tubulin exists in two isomers which dimerise to form rigid tubular structures approximately 25 nm ($\times 10^{-9}$) in diameter and may be several micrometers in length; their functions include facilitating substance transport, maintaining structural rigidity and participating in both nucleus and cell division [110].

3. **Intermediate filaments**. These constitute a very wide range of proteins whose distribution is not continuous between different cell types and will typically contribute to the specific functions of the cell. *P. polycephalum* and various other lower eukaryotes possess lamins, for example, which form a scaffold about the interior of the cell's nucleus to provide it with the mechanical stability it needs to replicate in the absence of a static cytoskeleton in a non-motile cell [133].

All three varieties of protein are bound together in one interconnected network via a range of actin and tubulin-binding proteins, the type of which determines the angle and conformation of the link made. For example, Arp2/3 complex forms branches on microfilament networks at approximately 60^o, whereas spectrin forms 'X'-shaped junctions between microfilaments [155].

Crucially, the cytoskeleton is both articulated onto a great many of the cell's internal components, including cell-surface receptors, nuclei and endoplasmic reticula[1], and is able to transmit a range of energetic and signalling events down its network.

Of these signalling events, the best-characterised is likely to be the ability of actin to transmit mechanical force from cell surface receptors to other regions of the cell; this increases the cell's robustness by reducing the force exerted on single areas, but has also been found to initiate a range of cell behaviours such as thigmotaxis (migration in response to tactile stimulation), thus indicating its role in signalling [155]. Furthermore, various cytoskeletal proteins have been demonstrated to transmit chemical signals via catalysis of second messenger pathways and vesicle transport, electrical potential in the form of ionic waves and quantum events such as minute thermodynamic vibrations (breathers), solitons and propagating waves of protein conformational changes [67, 82, 87, 118, 189, 193, 258, 307].

Thus, cytoskelton-mediated signalling is a prime target for UC research as it represents a network for spatial propagation and interaction of a wide range of data types. Furthermore, it also represents a tangible medium for structuring incoming data streams from receptors (sensors) and output chemical, electrical and mechanical data. It is for these reasons that the cytoskeleton was chosen as the medium upon which to base the functioning of the SMIPs developed during this period of research.

We are by no means the first to suggest that the role of cytoskeleton-mediated signalling is characterisable in the language of computation, but the vast majority of work to date on the topic focuses on the role of single microtubules as putative dual purpose data buses/individual logic gates. The first to comment on the computational nature of energetic events mediated by microtubules was Hameroff, who hypothesised in 1987 [137] that binaric processes involved in the cell cycle (e.g. alterations in the quantum characteristics of neural microtubules in response to the cell's depolarisation) may be the basis of consciousness when scaled-up in the billions of neurons within a brain. Although highly controversial in the early 1990's, Hameroff's work was complimentary to Penrose's influential theoretical work on the quantum nature of consciousness [231]; the two reserachers proceeded (and continue) to refine their theories in conjunction [138].

With regards to more computing-oriented research on the topic, Craddock *et al.* [82], continuing the earlier partly theoretical works of Lahoz-Beltra *et al.* [185], have suggested that conformational changes induced in the tips of microtubules resulting from

[1]The functions of this structure are, in cooperation with the Golgi apparatus, to package various macromolecules and ions in vesicles which are then tagged with surface proteins that dictate how they will be carried to their destination [41].

alterations in the electrochemical environment are equatable to Boolean logical operations. This phenomenon that has been linked to the formation and maintenance of memory in human neurons and has hence contributed to the hypothesis that a great many emergent phenomena exhibited by cells (including consciousness à la Hameroff and Penrose) are the product of non-linear interactions between intracellular signals mediated by the cytoskeleton [45, 86].

This 'cytoskeleton basis of emergent behaviour' is an attractive theory to court when debating the nature of apparently 'intelligent' behaviour patterns in brainless organisms such as *P. polycephalum*.

Our approach differs in two ways: firstly, we choose to focus upon actin as there are a wider range of signalling events that may pass through microfilaments and furthermore, actin network topology is highly dynamic and reforms constantly as the cell changes shape under mechanical stress or migration. Secondly, we recognise that any attempt to make conventional Boolean logical circuitry with cell components is fundamentally limited: to make an unconventional computing device, emphatically unconventional computing paradigms should be observed.

The *P. polycephalum* cytoskeleton has not been intensively researched, with the majority of studies focusing on its characterisation stemming from transmission electron microscopy projects in the 1970's [44, 332], but as our investigation in section 4.2 has demonstrated, the plasmodial actin network is sufficiently conserved between species such that a reagent marketed as being compatible with mammalian cells is adequately homologous to the slime mould equivalent that it was able to be incorporated into the *P. polycephalum* microfilament network.

This chapter is structured as follows: initially, the structure of the *P. polycephalum* actin network in various anatomical locations was sampled after which CA simulations were run in order to assess its viability for supporting information propagation and interactions. Following, we present laboratory experimental data characterising actin transmission of signal events as collision-based logical operations.

5.2 Excitable Cellular Automata Modelling of Slime Mould Actin Network Signalling

5.2.1 Introduction

The morphology of a data stream will determine the manner in which it functions. This investigation was performed in order to evaluate through the use of simple simulations whether plasmodial actin network topology is conducive to the transmission of and interactions between generalised energetic events. More specifically, as actin is considered to be a model excitable system [148] (see Appendix B.6), a basic excitable cellular automaton (CA) model was employed in order to simulate the transmission of generalised energetic events through experimentally-derived actin network graphs in order to indicate its properties as a data network and hence inform further steps into our investigations on cytoskeleton-mediated intracellular computation.

Network extraction was conducted using the Watershed transform, which has been previously demonstrated as accurately regenerating actin networks from microscopy data [117] (see Appendix B.8). Measurements made were integral excitation dynamics, speed of trans-network signal transmission and qualitative observations of signal transmission and interaction characteristics. As a secondary goal, these characteristics were compared in two different anatomical locations of the plasmodium — the advancing anterior margin and trailing caudal regions — in order to comment on how the organism's topological dimorphism may influence data network properties.

5.2.2 Methods

5.2.2.1 Slime mould cultivation and microscopy

Plasmodia of *P. polycephalum* were cultivated in the SSMME as described in section 4.2.2.2. Once the organism had propagated between the two agar hemispheres, the Petri dish was flooded with a fixative solution containing 2% paraformaldehyde and 0.1% glutaraldehyde in pH 7.2 potassium phosphate buffer for 1 h. They were then drained and rinsed in the same buffer three times for 10 m each. The organisms were then permeabalised with a 5 m treatment of 0.1% Triton X-100, after which they were rinsed again and stained with phalloidin conjugated to Alexa Fluor-488 (Molecular Probes, USA) for 1 h. After a final rinse, the SSMMEs were transferred to a confocal microscope and visualised as per the protocol described in section 4.2.2.2. All images were captured at the same magnification.

5.2.2.2 Network extraction and CA model

In order to represent the topology of actin networks in a 2D cellular automaton state space, actin network conformations were extracted from greyscale confocal micrographs using the Watershed Transform function in Matlab 2015a (Mathworks, USA). In the resulting binary images, in which the reconstructed actin network was black and everything else was white, only black cells were assigned as 'conductive', i.e. allowed to update their state. The concave hull of networks was also calculated for images where the cell's outer membrane was present in order to properly demarcate its boundaries.

In a generalised excitable medium, the state-space is discretised as a regular lattice of cells which may take one of the following three states:

Resting A stable state representing non-excited equilibrium.

Excited A cell becoming excited corresponds to its hypothetical energy level rising; in context, this equates to the section of excited actin network carrying an energetic signal.

Refractory Over time, an excited cell decays into a refractory state where it cannot be re-excited until a further amount of time has passed and it decays again into a resting cell. This corresponds to local depletion of energy.

The array was initialised by importing binarised 500×500 px bitmap images of actin network reconstructions: each pixel represented a cell, thus the CA interaction environment was a regular 500×500 lattice. Only the cells (pixels) corresponding to actin (black) were conductive, however, hence signals could only propagate through the actin network reconstruction. A resting cell becomes excited at time $t+1$, denoted by turning pink (see Electronic Supplementary Information in Appendix B.10), if it has at least one excited neighbour in its Von Neumann neighbourhood[2] at time t, hence signals may be observed to propagate in discrete time. Cells remain in an excited state for one time iteration before decaying into refractory (pink) for another time iteration, after which it re-assumes the resting state. When waves of excitation propagating along pathways of conductive cells collide, being flanked by refractory cells prevents their ricocheting or moving through each other and results in the annihilation of both signals.

[2]The Von Neumann neighbourhood refers to the number of cells in a regular two dimensional array that can influence another cell: this neighbourhood states that each cell has four neighbours; those above and below and those to the left and right. This is contrasted with a Moore neighbourhood in which the diagonals are included to make an eight cell neighbourhood.

Measurements made were integral excitation dynamics (the total number of cells excited during a given time iteration) as well as the speed (in number of timesteps) of transmission of a signal from the excitation point, a fixed, perpendicularly-oriented distance away, which was arbitrarily set at 450±1.2 pixels away in each. All experiments were performed 5 times for each sample.

5.2.3 Results

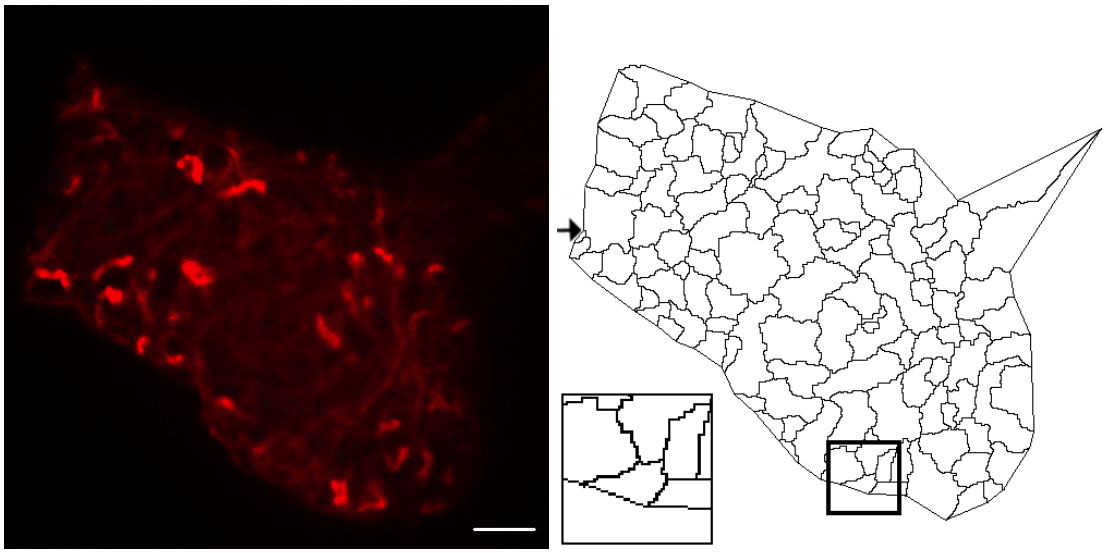
5.2.3.1 Imaging

In support of our observations in section 4.2.3, the *P. polycephalum* actin network was found to be dense and complex in the cortical (ectoplasmic) regions of plasmodial tubes and extended somewhat into the endoplasm (Fig. 5.2A). The cell's membrane also appeared to be intimately linked with its actin network. Networks were observed to be denser and more complex in the amorphous advancing anterior regions of the organism (Fig. 5.3A) than in caudal regions.

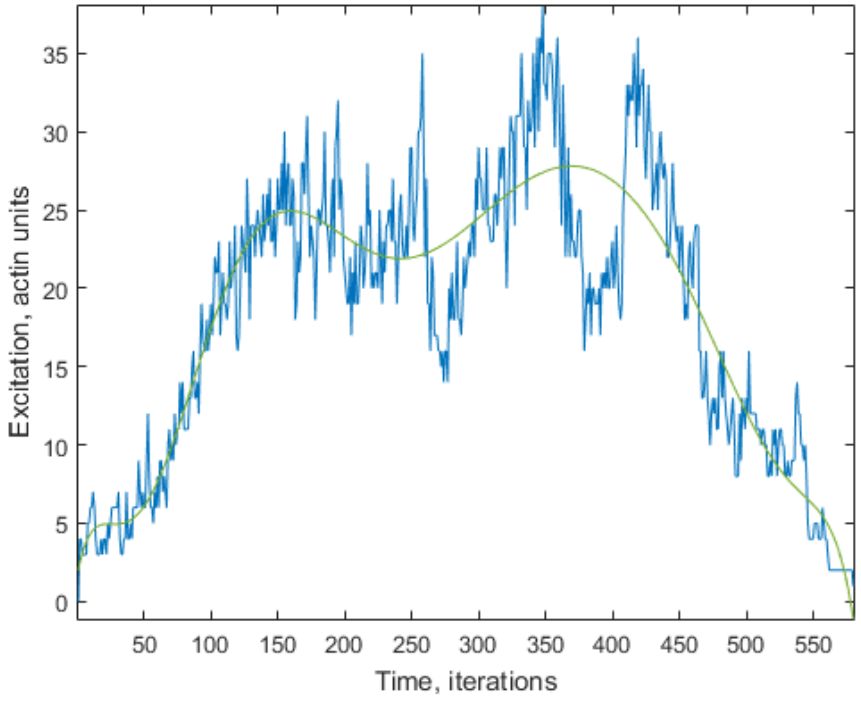
5.2.3.2 Network extraction and excitation dynamics

Watersheds from both a plasmodial tube and a section of advancing anterior margin are shown alongside their original images in Figs. 5.2B, 5.3B, video footage showing the time evolution of the CA model running through these networks is described in section B.10 and their integral excitation dynamics are shown in Figs. 5.2C and 5.3C. Both varieties of network were observed to support propagation of information from the excitation point to every other point in the network in all simulations. Amplification was frequently observed when signals interacted with network junctions (cells with three or more adjacent black cells), which was reflected in the richness of their excitation dynamics. Excitation dynamics were richer in the dense anterior margins of the organism. Signal back-propagation was only infrequently observed and when it was, errant signals were rapidly destroyed by collisions with other signals.

Signal propagation across a fixed distance was found to be faster in anterior margin actin networks in all experiments: in the examples given in Figs. 5.2 and 5.3, speed values (in timesteps) for each experiment were found to be 829 (range 789–1021, $n = 5$) and 659 (603–711, $n = 5$), respectively.

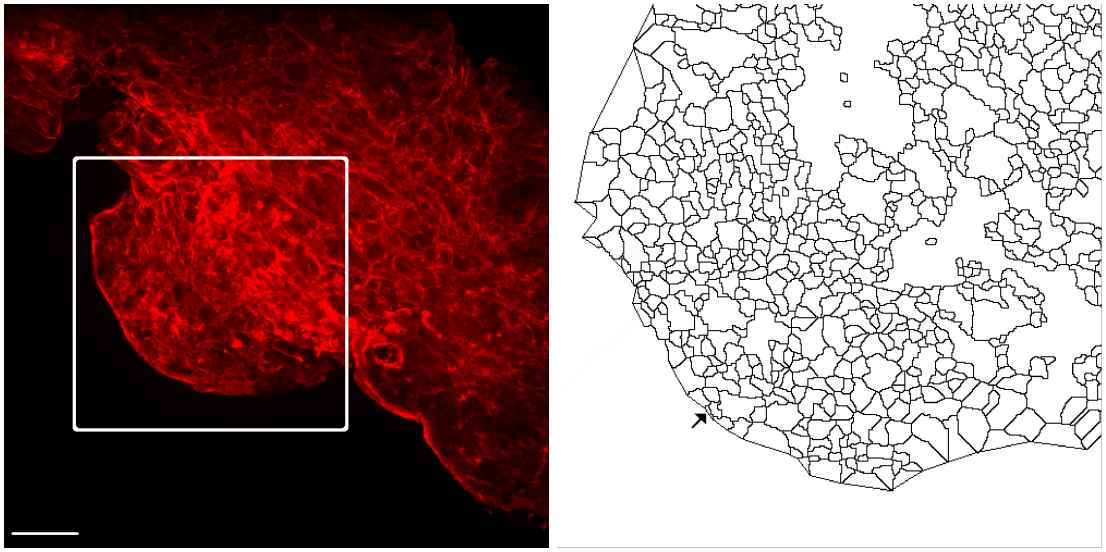


(a) (b)

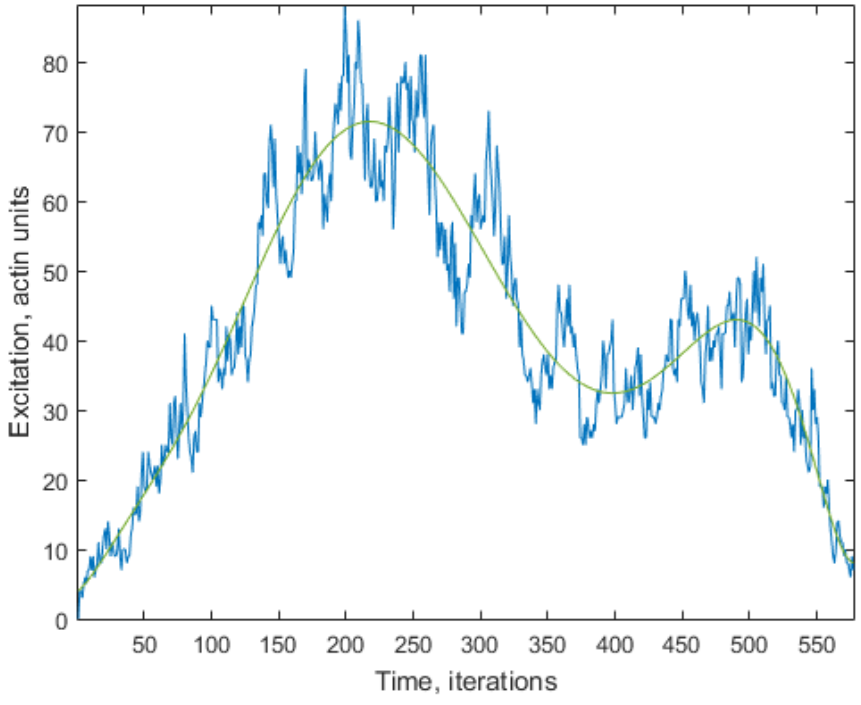


(c)

FIGURE 5.2: The actin network of a plasmodial tubule. (a) Confocal micrograph. Note the higher fluorescence returns around the cortical regions of the tube fragment. (b) Reconstructed network from [a]. Arrow indicates initial excited cell. The 50×50 px area indicated by a box is magnified in the lower left corner in order to indicate the relative sizes of the pixel lattice and the network reconstruction. (a–b) Scale bar = 50 μm. (c) Integral excitation dynamics with cubic spline. Adapted from the author's own work in Ref. [202].



(a) (b)



(c)

FIGURE 5.3: The *P. polycephalum* advancing margin actin network. (a) Confocal micrograph. The network is remarkably dense in comparison to Fig. 5.2A. White box denotes area analysed in [b]. (b) Reconstructed network from [a]. Arrow indicates initial excited cell. (a–b) Scale bar = 50 μm. (c) Integral excitation dynamics with cubic spline. Adapted from the author's own work in Ref. [202].

5.2.4 Discussion

Our results indicate that the plasmodial actin networks possess the following qualities which make them well-suited to supporting intracellular computation:

1. Generalised energetic events may propagate throughout the network without back-propagation, implying the ability for directional signal transmission.
2. Signals are amplified as they travel through branch points, indicating a high level of redundancy which is presumably beneficial in networks whose topology may spontaneously re-organise.
3. As actin network density is proportional to the speed and degree of amplification of a signal, we may speculate that the actin network may dynamically restructure to meet momentary physiological demands.

This latter point makes evolutionary sense, as the anterior margin of the organism must necessarily be required to output proportionally more sensing and actuation than trailing caudal regions, in which the network is reduced in order to save energy.

Excitable CA models are a popular method of simulating the behaviour of natural systems due to their comparative simplicity/ease of programming and capacity for generating complex behaviours from basic rules [128, 132]. As a generalised model, excitable CA share the basic characteristics of cells possessing either resting, excitable or refractory states; the system dynamics of the simulations presented here are accordingly fairly similar to those for other idealised excitable media such as reaction-diffusion chemical reactions or the propagation of action potentials in excitable cells [30, 175]. More specifically, their integral excitation dynamics are generally bell-shaped curves to correspond with the expenditure and depletion of reactants, which may oscillate to some degree during a medial plateau phase. The novelty of the simulations presented here is the introduction of an experimentally-derived interaction environment which allows for assessment of the state of the spatial computation with respect to the media topology in addition to gathering quantitative data pertaining to data propagation. To our knowledge, this approach has not been applied to biological systems, although selecting idealised interaction environments for cellular automata simulating purely chemical reactions (in reaction-diffusion media) has previously been performed [30].

To speculate as to the nature of the computational process within the *P. polycephalum* plasmodium, it has been demonstrated *in silico* that architectures based on large undirected planar graph topologies linking mobile data storage nodes may be used to implement general-purpose computation (Kolmogorov-Uspensky machines) [180]. Superficially, this would appear to be an attractive mechanism to describe how slime moulds are able to exhibit coordinated behaviours. For example, it is known that innervation of cell-surface chemoreceptors instantiates tip growth in the organism through momentary actin network assembly but it is unknown how the coordinated response of directional pseudopodium extension arises in the midst of every other chemical signal occurring on the cell membrane. We may speculate that, as the degree of stimulation of the cell's receptors is proportional to proximity to the attractant source, tip growth occurs at the correct site incident to the combined effects of greater degrees of signal amplification and destructive interference with other signals in the region. In essence, this is similar to a majority vote. This theory is an example of morphological computation as it implies that the organism is able to process countless (i.e. massively parallel) input signals and output a coherent behaviour without the need for synchronisation or centralised control: it occurs as a by product of signal interactions on the actin cytoskeleton.

Without examining specific actin-mediated signalling processes it is of course impossible to say how a practically useful actin-based intracellular computing device would function, but we note that the signal interactions observed in the excitable CA model presented are readily expressible in the language of computing. A signal splitting into two at a branch point is effectively a FAN-OUT function, whereas two signals destructively interacting at a branch point is expressable as $\langle A \cdot \bar{B} + \bar{A} \cdot B \rangle$, i.e. the XOR operation. This is, in effect, computation implemented under the Collision-based Computing (CBC) paradigm (see Appendix B.9 and following sections).

The model presented is not without its detriments: firstly, the images used were 2-dimensional (2D) representations of 3D structures and secondly, the structure of the organism's actin network is in reality in slow but constant flux. The data presented here are therefore used for illustrative purposes to suggest network characteristics and guide further study, although the simulation techniques used could be applied to 3D image stacks.

To conclude this section, we have identified that the *P. polycephalum* actin network is a viable medium through which processes characterisable as computation most likely occur and hence, it is a viable target computing resource to exploit in the generation of SMIPs.

5.3 Actin Automata

5.3.1 Introduction

Our excitable CA model indicated that the topology of the *P. polycephalum* actin network can support the transmission of energetic events and data–data interactions therein, but this model is unspecific and does little to tell us about the nature of physical signal transmission between protein molecules.

Unfortunately, it is currently impossible to empirically observe energetic events travelling through proteins on a molecular level in a live organism as the only techniques with suitable resolution, such as atomic force microscopy[3], require intensive sample preparation which requires that any cells used are killed, chemically fixed and cut into extremely small sections.

In the absence of an empirical method to probe the nature of actin-based computing, we instead present a comprehensive computational analysis of energetic event transmission through actin microfilaments with a bespoke semi-totalistic one-dimensional CA model configured to more accurately simulate actin dynamics than the generic excitable CA presented in section 5.2.

In efforts to avoid any confusion between this and the previous section, it is necessary to re-iterate that the CA presented here is emphatically different to the generalised excitable CA previously used and, accordingly, has a different dimensionality, state space and rule set.

5.3.2 Methods

When the g-actin monomers in a microfilament (Fig. 5.1) are considered as cells in a CA cell space, we find that each has 4 neighbours (Fig. 5.4), hence for a 5-cell neighbourhood (self plus neighbours) between there are 2^5 (32) separate rules[4] for a single chain and 2^{5^2} (1024) rules for the x and y chain. The neighbourhood of an actin automaton in the x chain x_i is $u(x_i) = \{x_{i-1}, x_{i+1}, y_i, y_{i-1}\}$ and for y_i in the y chain, $u(y_i) = \{y_{i-1}, y_{i+1}, x_i, x_{i+1}\}$. The state of an automaton, x_i or y_i are defined at each time step t as x_i^t or y_i^t.

[3] This technique uses a sub-nanoscale electrical probe to concurrently observe and electrically characterise a substrate

[4] 'Rules' here referring to the conditions (number and position of excited neighbouring cells) which will cause a cell to change its state, in the Wolfram notation. Rules are binaric as it distinguishes only between excited and non-excited, hence there being only 2^5 rules per chain, as opposed to 3^5.

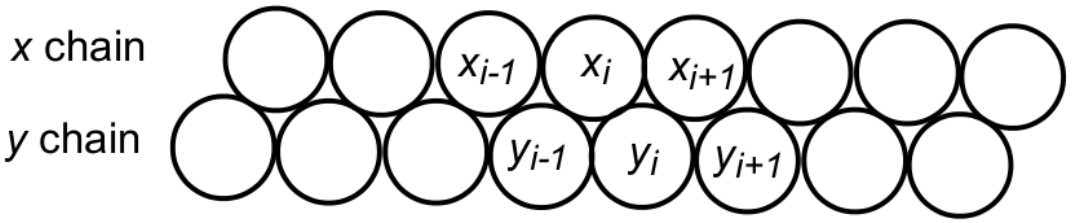


FIGURE 5.4: Schematic diagram of an actin microfilament showing the neighbourhoods for g-actin molecules in both chains. Adapted with permission from Ref. [34]

The model constructed was semi-totalistic in that the centre cell polls its neighbours and assumes the state of the majority value: as such, let $\sigma^t_{x_i} = x^t_{i-1} + x^t_{i+1} + y^t_i + y^t_{i-1}$ for the x chain and $\sigma^t_{y_i} = y^t_{i-1} + y^t_{i+1} + x^t_i + x^t_{i+1}$ for the y chain be the sums of excited neighbours. Cells update their states according to a transition function $F = (f_{i,j}, 0 \leq i \leq 1$ and $0 \leq j \leq 4, f_{i,j} \in \{1, 0\}$ in discrete time as $x^{t+1} = f_{x^t_i, \sigma^t_{y_i}} / y^{t+1} = f_{y^t_i, \sigma^t_{y_i}}$.

Rules were represented as decimalisations of binary cell configurations in the Wolfram notation [335], e.g. rule (8,12) would equate to $F_0 = (01000)$ and $F_1 = (01100)$. Experiments were run for every rule combination with random starting configurations (probability of 0.5); for n automata evolving for τ timesteps, typical values for each experiment were $n = 300$ and $\tau = 1000$. Data were collected as a two-dimensional matrix $M = (m_{i,j})$, where $m_{i,j}$ is automaton i state at timestep j, $1 \leq i \leq n$ and $1 \leq j \leq \tau$. The following integral measures were chosen to characterise the CA's complete rule space due to their previously having being demonstrated as suitable for complete characterisation of CA systems [17, 18, 28]:

- **Shannon entropy** H. Also known as information theoretical entropy, this measurement is used to estimate the minimum bit length it is possible to encode a string into; this gives an estimation of the informational capacity of the system. W is a set of all possible configurations of a 9-cell neighbourhood in the form (i, j) of matrix M. The number of non-resting configurations (i.e. non-static patterns) $\eta = \sum_{a \in M} \epsilon(a)$ was calculated, where $\epsilon(a) = 0$ when all the neighbours of a pattern a are resting, and $\epsilon(a) = 1$ otherwise. The Shannon entropy is calculated as $-\sum_{w \in W}(\nu(w)/\eta \cdot ln(\nu(w)/\eta))$, where $\nu(w)$ is a number of times the neighbourhood configuration w is found in matrix M.

- **Space filling** P. A ratio of non-resting nodes in space time configuration of $n \times \tau$ of entities in a given array, this measurement represents the proportion of the final planar graph that is shaded. $P = \sum_{1 \leq i \leq n, 1 \leq j \leq \tau} m_{ij}$.

- **Activity** A. A quantitative measure of the degree to which the array becomes filled with excited cells. $A = (\sum_{1 \leq i \leq n, 1 \leq t \leq \tau} x^t_i) \cdot (n \cdot \tau)^{-1}$.

- **Incoherence** I. The difference in activity between x and y chains. $A = |\sum_{1\leq i\leq n, 1\leq t\leq \tau} x_i^t - \sum_{1\leq i\leq n, 1\leq t\leq \tau} y_i^t| \cdot (n \cdot \tau)^{-1}$

The following methods were adopted to isolate rules supporting the generation of travelling localisations. Resting x and y chains 5 molecules in length were excited with seeds $X = (x_0, \ldots, x_4)$ and $Y = (y_0, \ldots, y_4)$ such that the evolution of an actin automaton from seed $s = \langle X, Y \rangle$ over τ timesteps was measured. If the activity of the resulting plot was $10 \leq A \leq 6 \cdot \tau$ then seed s was reasoned to generate either travelling or stationary localisations.

5.3.3 Results

5.3.3.1 General features

Superficially, no characteristic differences were observed between x and y chain patterns (Fig. 5.5).

Rules generating lower H values (< 1.8) were found to generate solid patterns of either resting or excited cells. Conversely, rules with H values between 1.8 and 4.3 consistently generated ordered patterns of excitation and those with values exceeding 4.5 displayed irregular, pseudo-chaotic behaviour (Fig. 5.6).

The excitability of simulated g-actin molecules, that is likelihood to transition from state $0 \mapsto 1$ or $1 \mapsto 1$ based on the number of excited neighbours, was found to be proportional to H: this was calculated by splitting the H rankings of the configurations generated into 50 classes $L = L^1, \ldots, L^{50}$, 0–5 in 0.1 increments. The frequency vectors G^z for states $0, 1$ were then calculated according to $G^z_{ki} = |L^z|^{-1} \cdot \sum\{F_{0i} : F_0 \in L^z\}$, $k = 0, 1$, where $|L^z|$ is the size of class L^z. In plainer terms, the frequency vector indicates how often the excited state appears in vectors of F_k, $k = 0, 1$ for each class of entropy and normalised against the size of each class. As Fig. 5.7 demonstrates, plotting these vectors allows us to deduce the frequency vector (i.e. number of excited neighbours) most likely to result in a cell's excitation verses entropy values. Our results indicate that at lower entropy values, the $0 \mapsto 1$ transition is most likely when 4 neighbouring molecules are excited and $1 \mapsto 1$ when 3 neighbours are excited: as entropy values rise, the ideal number of neighbouring cells required to generate or maintain an excited cell falls.

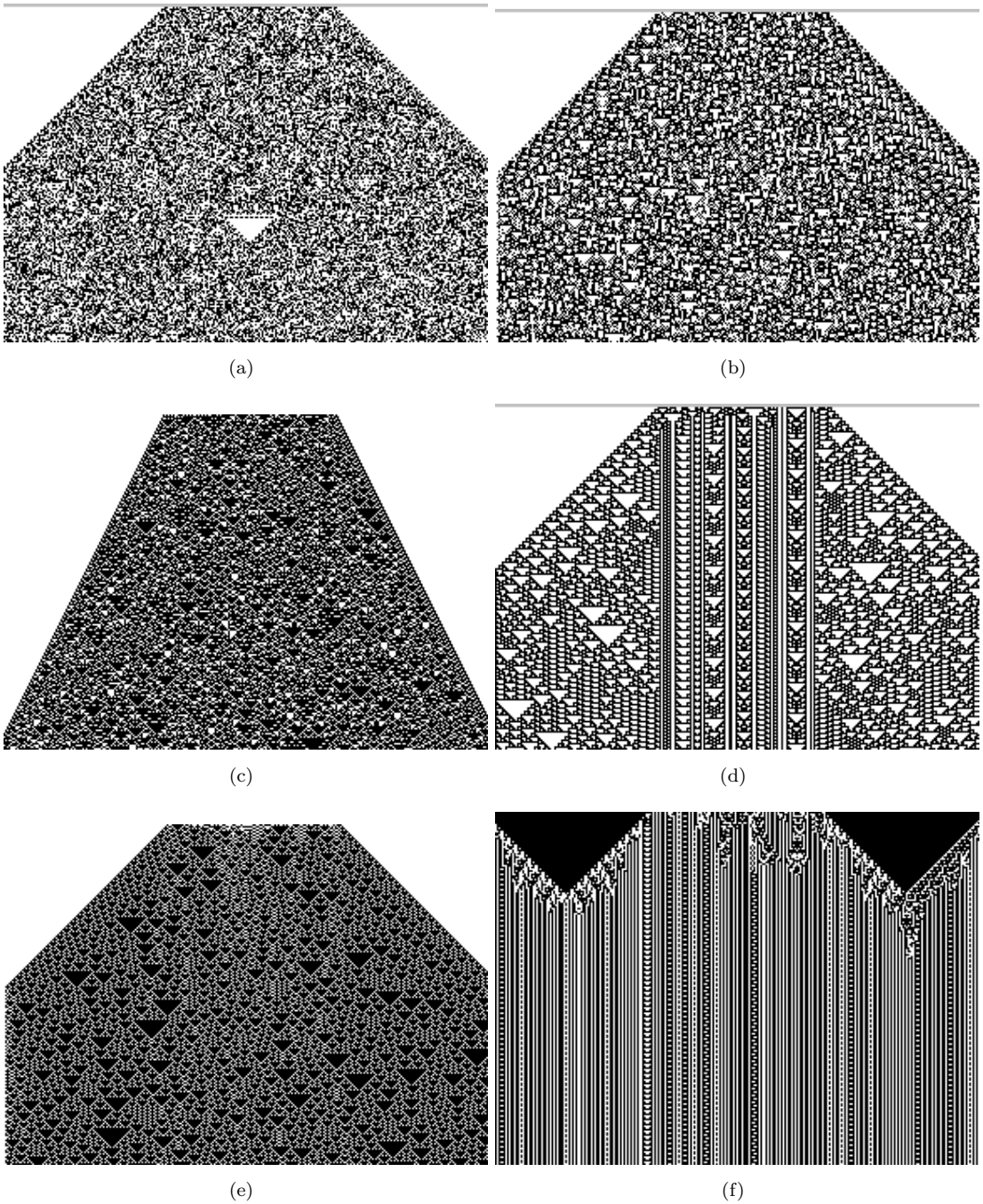
5.3.3.2 Generation of localisations

Approximately 30% of rules were found to support the generation of localisations; those that did tended to have higher entropy. As such, the ability of a rule to generate high

FIGURE 5.5: Spacetime evolution of actin automaton, rule (4,25). Time goes down. (a) x chain. (b) y chain. (c) x, y incoherence. Adapated from the author's own work in Ref. [34].

numbers of localisations is correlated with actin excitability. The top 10 rules, expressed as richness of localisations arising from individual seeds T are shown in Table 5.1.

Rules supporting high numbers of localisations also typically had low activity and a high degree of space filling. Both types of localisation, stationary (travelling straight down) and travelling (travelling diagonally) were observed, but not all rules supporting localisations exhibited both varieties. Collisions between localisations were observed frequently and could result in either the destruction of both signals or the generation of new travelling localisations (Fig. 5.8).

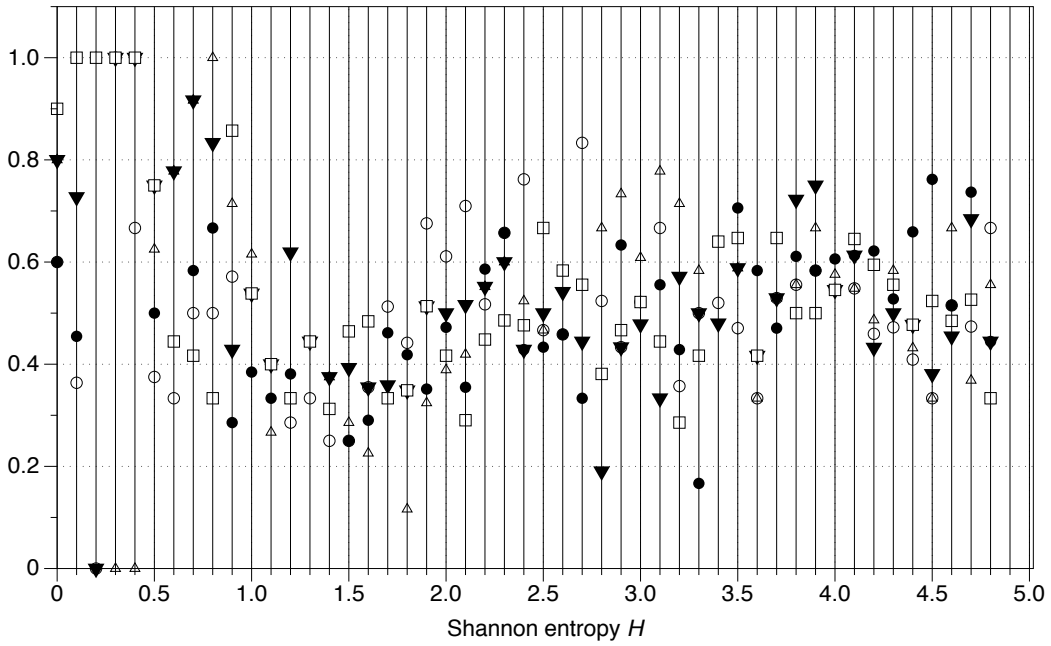


(a) (b)

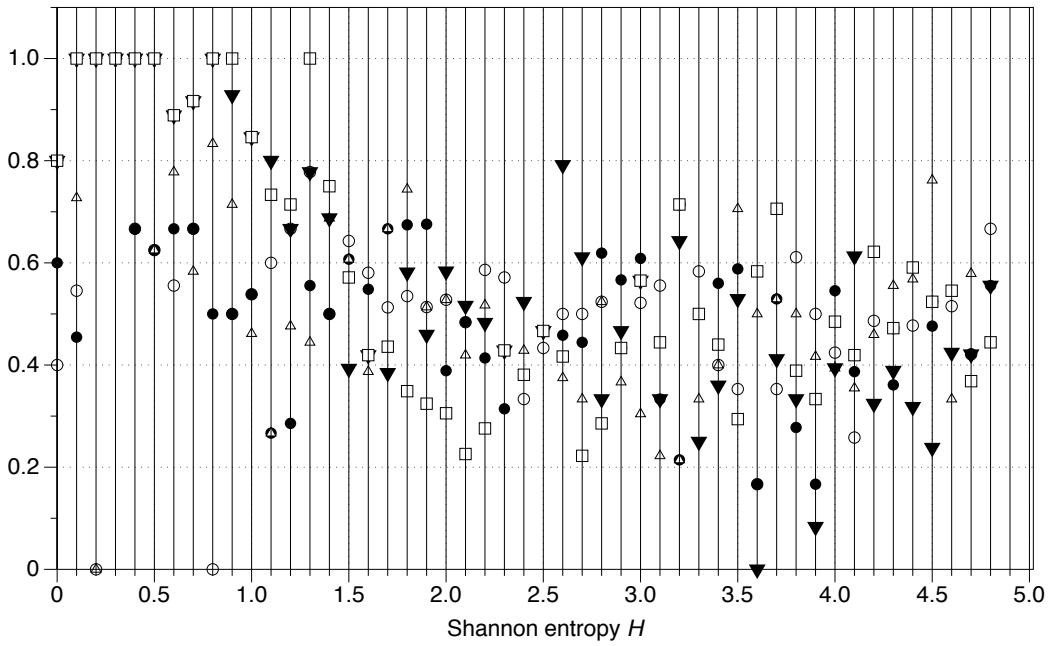
(c) (d)

(e) (f)

FIGURE 5.6: Exemplar spacetime configurations developed in the x chain from random initial configurations in 100 nodes for the following rules and H values: (a) (10,10), 4.8. (b) (11,6), 4.5. (c) (7,29), 4. (d) (11,14), 3.5. (e) (14,9), 3. (f) (20,13), 2.5. Adapted from the author's own work in Ref. [34].



(a)



(b)

FIGURE 5.7: Graphs to show frequency vectors vs. Shannon Entropy H. (a) Vector G_0 representing the $0 \mapsto 1$ state transition. (b) Vector G_1 representing $1 \mapsto 1$, i.e. maintenance of excited state. Empty circle = no excited neighbours; solid circle = one excited neighbour; upwards triangle = two excited neighbours; downwards triangle = three excited neighbours; square = four excited neighbours. Adapted from author's own work in Ref. [34].

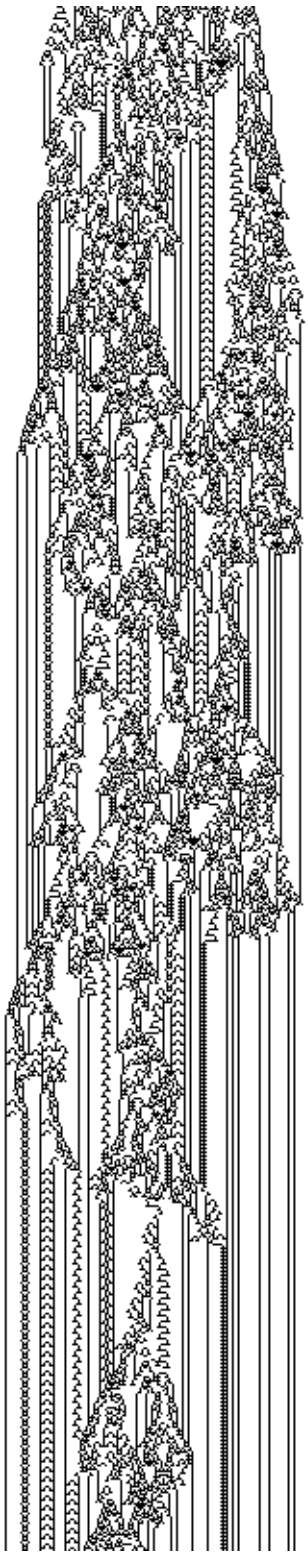


FIGURE 5.8: Spacetime configuration for rule (5,25) that was found to generate both travelling and stationary waves. Collisions between localisations are frequent. Adapted from the author's own work in Ref. [34].

Rule	T	H	P	A	I
$(7, 20)$	271	4.31	0.84	0.32	0.28
$(5, 26)$	240	4.70	0.92	0.39	0.39
$(4, 26)$	210	4.54	0.86	0.31	0.34
$(5, 25)$	197	3.83	0.73	0.14	0.19
$(6, 20)$	197	4.37	0.86	0.37	0.28
$(8, 0)$	191	3.50	0.83	0.23	0.26
$(7, 21)$	179	4.31	0.97	0.56	0.39
$(8, 1)$	170	3.50	0.84	0.23	0.26
$(8, 24)$	146	3.71	0.80	0.25	0.18
$(7, 4)$	145	2.75	0.54	0.06	0.07

TABLE 5.1: Table to show localisation richness T and time integral characteristics for the actin automata rules supporting generating the most localisations. Adapted from the author's own work in Ref. [34].

5.3.4 Discussion

We observed that the entropy of actin automata is in general proportional to the sensitivity of actin molecules to excitation by neighbouring units, but that over-excitation (having too many excited neighbours) of an already excited molecule can reduce the likelihood of signal propagation. This equates to a transition from the system supporting excitations/amplifying weak contrasting signals (lateral excitation) to actively inhibiting them at times of excessive excitation (lateral inhibition). This is of particular interest to us as these are defining characteristic of neural networks and are thought to give rise to emergent behaviours such as colour discrimination and fine control of tactile manipulation [342]; although this is contextually only a casual link, this nevertheless demonstrates that actin automata can give rise to complex computing characteristics within all eukaryotic cell types.

Higher-entropy rules were found to support the generation of both travelling and stationary localisations to a proportional degree: coupled with the phenomenon of lateral inhibition in over-excited molecules, we may therefore speculate that there is an ideal excited neighbours ratio for a microfilament to support the generation of localisations, the number of which may vary depending on the excitability of the medium.

Although some have previously advanced generalised CAs in order to describe actin assembly dynamics [103], the variety of CA presented in this chapter is bespoke — i.e. designed to specifications that mimic energetic transfer between actin molecules — and as such, the novelty in our results lies within the insights into spatial computation in actin we have gathered; excitability, XY incoherence etc. Our work has since been enhanced by Alonso-Sanz and Adamatzky [47], who adapted the CA such that each actin molecule/cell has the ability to remember its previous state; actin automata with memory display slower propagation and generate less complex (lower entropy) patterns.

The incidence of collisions between travelling:travelling and travelling:stationary localisations indicates that the medium is suitable for implementing computation via Fredkin and Toffoli's collision-based computing (CBC) model (see Appendix B.9 and section 5.4), as collisions between spatially-propagating objects in CA models have repeatedly been demonstrated to be viable computing media [7–9].

Let us attempt to apply some form of biomolecular interpretation to this abstract model. Firstly, from morphological analysis of spacetime evolution plots of the various rules of note presented here that generate localisations, we may observe that they are best described as class 4 rules under Wolfram's classification [336], i.e. that complex mixtures of stable, oscillating and pseudo-chaotic patterns evolve over time. Whilst care must be exercised in any such subjective, qualitative interpretations[5], we may nevertheless state that we have observed 'emergence' in our simulated system. Secondly, we may begin to link specific signalling events to the model.

For example, actin is a strongly charged molecule which acts as a polyelectrolyte (absorbs anions from its medium). F-actin microfilaments have been demonstraetd to conduct ionic waves as nonlinear solitary waves (solitons) that travel through its constituent molecules as a propagating cascade of alterations in molecule charge, accompanied by a complimentary coherent wave of counterions passing through the neighbouring cytoplasm [189, 307]. This phenomenon has been suggested to be a means of data transmission and processing in the mammalian neural cytoskeleton [337]. Thus we may speculate that the information transmission events modelled with our CA model equate to this mechanism, where:

1. Lateral excitation equates to transmission of charge down the gradient between two neighbouring actin molecules.
2. Lateral inhibition is represented by ionic depletion in the buffer of the local cytoplasm.
3. Processing occurs when two travelling localisations (solitons) collide.

To conclude, our suggestions for further study are summarised below.

1. x, y incoherence spacetime plots are morphologically interesting and represent an unexplained dysjunction between the two chains, but it remains to be seen if they hold any value in representing the behaviour of the automaton. Further work

[5] Although, note that certain fields of academic study such as those based on microscopical sciences (comparative anatomy, histopathology etc.) are based on such observations under the protective banner of 'strength in number of observations'.

could involve exploring any correlations that may exist between incoherence and novel quantitative measures of automaton behaviour, such as compressibility or diversity.

2. Although the model presented here is exhaustive with regards to characterising signals on single microfilaments, it does not represent microfilament networks with branches or intermediate actin-binding proteins, the properties of which are likely to be very different and may perhaps be better approximated with statistical methods: this could be a focus for further research, although model complexity would doubtless prove restrictive.

5.4 Cytoskeleton-mediated Collision-based Computing with Calcium-filled Vesicles

5.4.1 Introduction

Following the theoretical and simulational underpinnings detailed in previous sections of this Chapter indicating that further study into cytoskeletal computing would be worthwhile, this final section in our investigation into the development of SMIPs concerns an experimental study in which we characterise a slime mould actin network process as computing operations.

Our previous results have indicated that a range of generalised cytoskeletal signal transmission events should be based on the principle of spatial propagation of information and also that the CBC paradigm is of great use in implementing computation with such a representation of data. Inspired by Margolus' Soft Sphere Modification (SSM) of the BBM [196], in which the archetypal billiard balls are replaced by pliable spheres which compress on impact and travel as a single object for a certain period before rebounding (moving away from each other), we designed the following investigation into the characterisation of a slime mould intracellular system as a CBC-derivative computing device.

More specifically, we define intracellular quantities of calcium held in phospholipid membrane-bound sacs (vesicles) as data, both for their properties as discrete localisations within their cell as well as their physiological function, which is to sequester calcium for its transport through the cell prior to its release as a component of the cell's biomechanical oscillator. As such, calcium is an ideal analogue of biological 'information' as it is released in response to stimuli originating from the environment and has measurable, repeatable effects on the cell when it reaches its target destination, i.e. the

data is structured. Indeed, others have previously suggested that calcium is a suitable signalling molecule for use in excitable chemical processors, although all work to date in this area is theoretical and based on reaction-diffusion concepts [106, 145]. For the purposes of this investigation, however, output is interpreted in the manner of the original CBC and SSM models, i.e. an operation is interpreted as TRUE if the vesicle follows a specific, pre-determined path.

Crucially, calcium-filled vesicles (CFVs) are known to articulate onto and travel down the actin network of mammalian, protist and plant cells and directional actin-mediated transport of vesicles through the cell is a well-characterised phenomenon [259, 293]: this long-range cellular transport mechanism is achieved via vesicle coupling to myosin II in the endoplasmic reticulum, through which they gravitate towards and attach to the microfilament network. Myosin physically 'walks' the attached vesicle along each monomer of the microfilament in the direction of the cell's periphery (as dictated by the microfilament's polarity), driven by ATP hydrolysis [51, 281, 293]. This provides a completely-defined biomolecular explanation for the generation of travelling localisations.

5.4.2 Methods

Stock cultures of *P. polycephalum* plasmodia were cultivated as per the protocol in section 3.1.2.1. Experimental plasmodia were then grown as per the SSMME. When the organism had propagated between the two hemispheres of NNA, the plasmodial tubule linking them was injected with approximately 1 μl of a solution containing a fluorescent calcium indicator using a CellTram microinjection system (Eppendorf, Germany) with a hollow glass needle, tip < 30 μm. One fluorimetric indicator, Calcium Green-5N (Life Technologies, USA), 488nm excitation, at a concentration of 1 mM, and one ratiometric indicator, Fura-2 (Life Technologies, USA), 405nm excitation, at 5 mM, were used, which were both prepared in distilled water. Confocal imaging and post-processing was performed as per section 4.2.2.2, with the addition that gain was adjusted through software-based adjustment of the CCD camera's electron multiplier in order to filter out background calcium levels. The cytoskeleton was not labelled as staining methods typically disrupt normal patterns of cytoskeletal activity.

5.4.3 Results

Calcium was observed to exist in microscopic spherical quantities, as expected, whose sizes were inconsistent but usually between 3–7 μm in diameter. These objects were

observed travelling through the cytoplasm at a velocity of approximately 5 μm s^{-1}; directionality and vesicle speed were not consistent with shuttle streaming, but their paths were observed to follow well-circumscribed routes through the organism, i.e. multiple vesicles were observed to travel through exactly the same routes. Vesicles were observed more frequently in the plasmodial ectoplasm and anterior margin. Collisions between quantities of calcium were frequent but there were multiple varieties of collision outcome; the types and frequency from a sample of 42 observed collisions are summarised here (Fig. 5.9):

I **Reflection**: 57.1%. Two vesicles collide and reflect, after which their incident paths diverge from their apparent initial direction.

IIa **Fusion, adhesion**: 9.5%. Both vesicles merge on collision but appear to be two separate objects adherent to each other, which may or may not dissociate after any length of time.

IIb **Fusion, assimilation**: 14.3%. As with type IIa, but both vesicles appear to form a single structure which is larger than either of the two original objects. Assimilated vesicles do not dissociate.

III **Annihilation**: 9.5%. Following a collision, both vesicles appear to rapidly disperse their contents into the surrounding cytoplasm.

IV **Unknown**: 9.5%. No observed outcome despite the fluorescence returns for both vesicles coming into contact with each other.

In instances where the outcome of a collision was unclear due to the objects moving out of the microscope's field of view, the event was discounted. Quantities of calcium larger than 7 μm were also discounted as no measures were taken to distinguish between endoplasmic reticulum and vesicles. Exemplar microscopy footage is included as Electronic Supplementary Information (Appendix B.10).

5.4.4 Discussion

5.4.4.1 Identification of vesicles and collision classification

The quantities of calcium observed were identified as CFVs by their size in comparison with transmission electron microscopic studies into their identification [6, 183], elastic interactions, patterns of distribution and tendency to travel down certain paths through the organism; with regards to this latter point, these pathways were assumed to correspond to networks circumscribed by the organism's actin network.

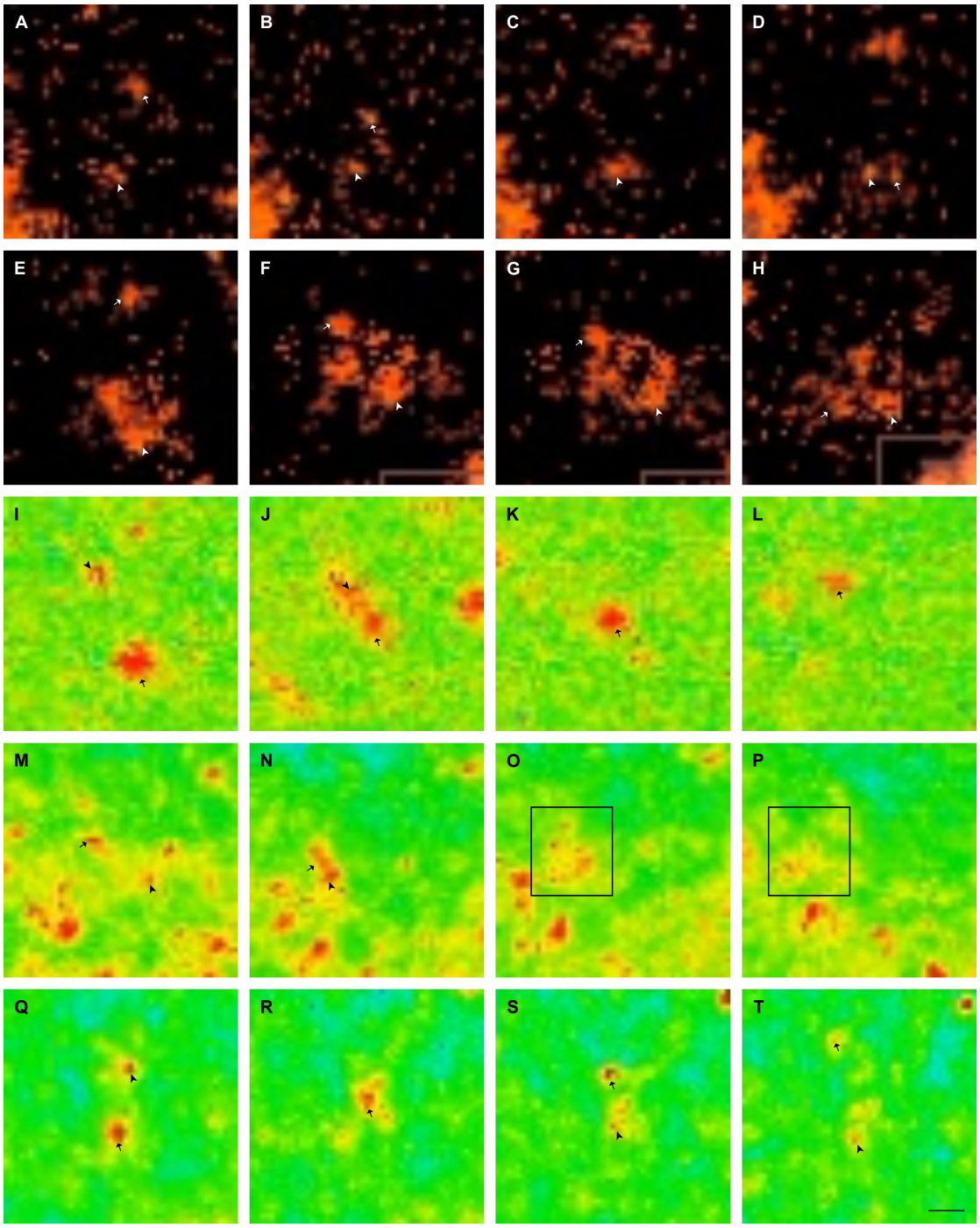


FIGURE 5.9: Freeze-frames from confocal microscopy video footage from plasmodia microinjected with fluorescent calcium dyes to show types of vesicle collision. Arrows and arrowheads indicate two colliding vesicles (a–d) TI, reflection. (E–H) TIIa, fusion adhesion. (I–L) TIIb, fusion assimilation. (M–P) TIII, annihilation. Box indicates region in which the annihilating vesicles dissipate. (Q–T) TIV, unknown. (A–H) Fura-2. (I–T) Calcium Green-5N. (A–T) Scale bar = 10 μm, time steps approx. 250 ms. Adapted from the author's own work in Ref. [205].

5.4.4.2 Computing with vesicle collisions

We propose that CFVs are viable computing media for a modification of the BBM that we shall call the 'Vesicle Collision Model' (VCM). Consider a generalised microfilament network: within the VCM, the plasmodial actin network is likened to the gridlines of a Euclidean plane in the BBM such that our quantities of data may travel along them. CFVs are loaded onto the network in a variety of locations but all travel in the same direction, as dictated by the microfilament's polarity. Collisions occur when two vesicles meet following a branch point in the network, the outcome of which represents a logical operation.

We were not able to isolate the factors which determine collision outcome, but these could include the size/quantity of calcium within each vesicle relative to a critical capacity, membrane-bound proteins which influence vesicle behaviour or/and the angle and net velocity of collision. The predominant collision type, reflection, holds the most resemblance to the SSM as CFVs undergoing this variety of interaction appear to deform for 50–100 ms prior to reflecting, the cause of which is presumably elastic recoil. It is assumed that recoiling CFVs remain bound to the cell's actin network but divert to a different branch (as indeed the actin network's redundancy would suggest that multiple branches will still lead to the same general destination), although it is not outside the realms of possibility that one or both detach. It is eminently possible to confine all VCM paradigms to type I collisions due to their similarity to existing paradigms, especially if vesicle collisions could be engineered to favour this variety of collision.

Type II and III collisions are somewhat more difficult to equate to previously-described modes of CBC as they are not conservative (although technically neither are type I; see following sections), but that is not to say that they are without function: type IIa collisions could be regarded as a delay element, for example, whereas type III could be regarded as a 2-to-1 FAN-IN/STOP operation. Furthermore, type III collisions are of particular note if one considers the possibility that the physiological purpose of the collision mechanism may be to achieve local un-loading of CFV contents. In the language of computing, this links the computation directly to a tangible output.

It would appear that type IV collisions are not actually true collisions but are instead the result of two vesicles passing closely rather than colliding, possibly in the z-axis; this highlights the difficulties of measuring or co-localising objects with fluorescence imaging as object size is distorted by probe fluorescence intensity and relative concentration.

5.4.4.3 Logic, bijectivity, entropy and viability of biological substrates in the VCM

Type I collisions can be characterised as a realisation of Fredkin and Toffoli's BBM interaction gate, which is a reconfigurable gate that is most commonly considered to complete the AND function such that $\langle A \wedge B \rangle = 1$ (Fig. 5.10), but it may also be viewed as a variety of other gates depending on how the output is interpreted. Crucially, despite carrying out a binary logical operation, the interaction gate exemplifies the UC paradigm of input-output bijectivity as the quantities of data are conserved.

Care must be exercised with the use of such terms, however, as any logical gates we may propose based on these phenomena are emphatically not conservative: indeed, none of the few experimental prototypes of CBC circuits fabricated to date are [9, 10, 136] as it is exceptionally difficult to fabricate an isentropic device in practice. In plainer terms, Fredkin and Toffoli's original designs for BBM circuitry were fully reversible (a beneficial property for a computing device; see Appxendix B.9), deterministic systems by virtue of being non-dissipative; whilst this appears on face value to be a casual design for a perpetual motion machine, the paradigm's creators emphasised that CBC circuits have no *internal* dissipation of energy [122]. In using this wording the authors were referring to a physical system characterised by n degrees of freedom split into $n = a, b$, where a are the 'mechanical', highly ordered modes which obey strict physical laws (and are hence deterministic and reversible) and b are the 'thermal' modes to which waste energy is dissipated from the mechanical modes: there are typically far more thermal modes than mechanical in order to achieve efficient transfer, but this transfer from one to the other gives rise to chaotic, nondeterministic behaviours. In an ideal CBC system, mechanical modes interact with each other, hence they are isolated from the influence of thermal modes and there is no 'internal' dissipation of energy. In real-world systems, however, the number of degrees of freedom of a system and hence thermal modes is equivalent to Avogadro's number (the number of molecules, $6{\times}10^{23}$, per mole of a substance). Our slime mould system consumes energy in the form of ATP via its hydrolysis, and a certain amount of the energy from this is dissipated as heat, which therefore leads to chaotic behaviours under the physical definition of the term. But how are organisms able to display any variety of coherent, repeatable behaviour if the physics of their underlying biomolecular processes are inherently chaotic?

We present the hypothesis that biological substrates are a viable class of media for implementing CBC-derivative paradigms, despite their inherently irreversible mechanisms because of damping, a phenomenon alluded to in Fredkin and Toffoli's original treatise [122] (but not with regards to novel computing media) which refers to a system that maintains an internal energy equilibrium that greatly favours the mechanical modes.

Thus, its macroscopic behaviours are inherently repeatable and predictable as they are governed almost exclusively by reversible processes. To return to our previous findings pertaining to morphological computation concepts, behaviours are kept repeatable as a result of information structuring via cytoskeleton-mediated processes; these are self-evidently governed by strict physical laws and hence constitute mechanical modes. Furthermore, we recognise that the ability of an organism to maintain a homeostatic equilibrium is a biological realisation of the principle of damping, i.e. an energy gradient to the mechanical modes is maintained through energy production (metabolism) and signal regeneration, and thermal transfer is kept to a minimum thanks to the evolution of supremely energy efficient life processes [277]. Thus, under the tenets of our hypothesis, the naturally-evolved systems for energy efficient manipulation of somatosensory data streams possessed by biological substrates such as our model organism exhibit inherently programmable behaviours.

Thus, adjusting our designs for logic gates to include non-conservative CBC designs, we find that a huge variety of logic devices may be conceived of. To illustrate this point, consider that a type III collision's output may be considered as an XOR gate, $\langle A \wedge \bar{B} \rangle \vee \langle \bar{A} \wedge B \rangle$, due to the annihilation of both signals when the input is configured as $\langle A \wedge B \rangle$. AND and XOR constitute a computationally universal[6] set of Boolean operations, albeit achieved through an emphatically unconventional construct.

5.4.4.4 Towards practical VCM computing

Whilst our results represent the first intracellular realisation of CBC and, debatably, the first scheme for the operation of a SMIP, it is clear that a certain amount of control needs to be exerted over these mechanisms if they are to be used for true practical benefit. This section details our designs and suggestions for a practical implementation of VCM computing.

One route towards implementing an *in vivo* VCM system would be some form of machine wherein the outcome of a calculation (its collision type) is bounded by the probability of that type of collision. This would require the minimum of engineering as such a device would require only a means of stimulating vesicle transport and automatically interpreting microscopy footage, after which the responses of the system would need to be statistically characterised. That said, the field of computing with probabilistic processes is not without its detriments: indeed, some have noted that the problem of defining complex systems in terms of probability trees leads to extremely complex

[6] This term is used here simply to denote that these two gate varieties can be used to make a functionally complete set of Boolean logical gates, rather than referring to the computation universality principle.

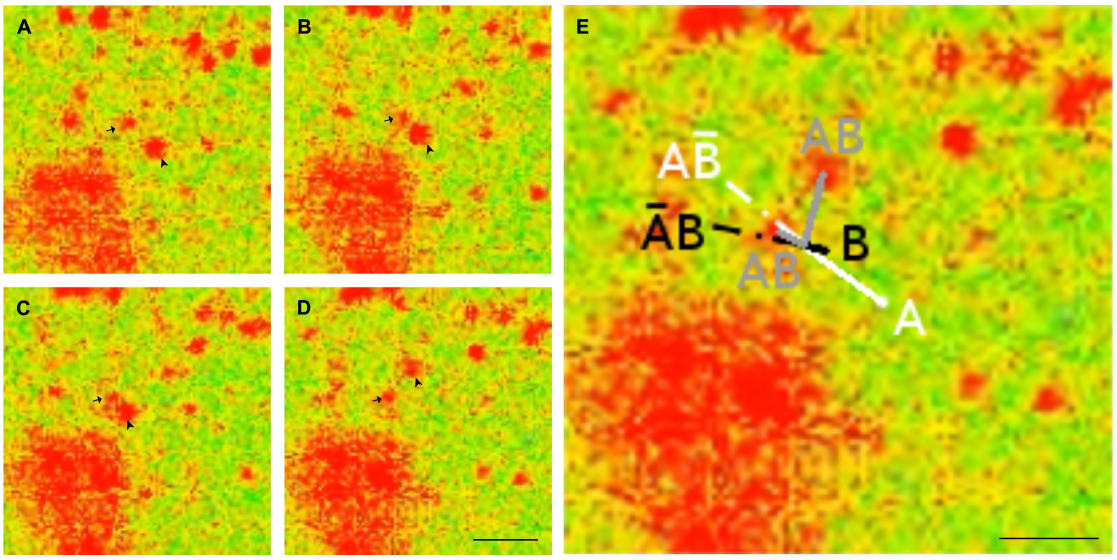


FIGURE 5.10: Confocal micrographs demonstrating how a type I collision may be characterised as an interaction gate. (a–d) TI collision corresponding to the input configuration $\langle 1,1 \rangle$. (e) Enlarged frame from [D] showing vesicle start points (A,B), original trajectories (dashed lines) had the input configuration been $\langle 0,1 \rangle$ or $\langle 1,0 \rangle$ and the paths of the vesicles following their collision such that an AND operation was completed. (a–d) Time steps approx 250 ms. Scale bars = 10 μm. Adapted from the author's own work in Ref. [205].

constructs whose monolithic scale makes performing the calculations designed for them (Bayesian inference, for example) computationally intractable — at least when run on conventional architectures [194].

The alternate approach would be to engineer collisions on networks with a desirable, ideally pre-determined topology such that more conventional CBC collisions could be implemented. This presents the necessity to:

1. Fabricate actin networks with a known topology, or at least utilise common conformations extracted from networks in live cells.
2. Load vesicles onto said network at specific points such that their motion can be synchronised.
3. Define collision type (if this is not determined through point 1).
4. Enable some form of output detection.

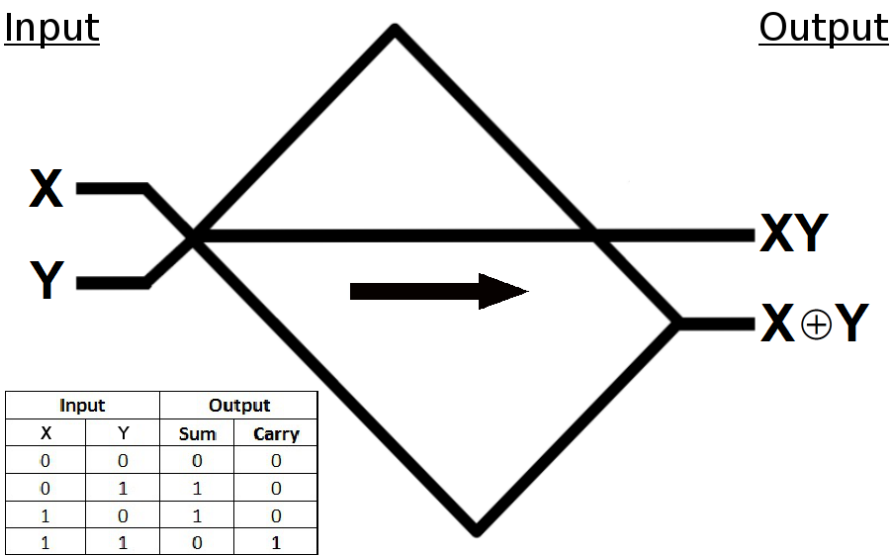
These requirements are not as insurmountable as they may seem. Minute manipulation of cytoskeletal proteins has been achieved *in vitro* [189] and actin network growth is essentially programmable as its assembly is entirely determined by the presence or absence of a few actin binding/nucleating proteins and a suitable physiological medium [155].

Furthermore, actin networks may only grow in a few specific conformations as all forms of network articulation are governed by actin binding proteins: Arp2/3, for example, mediates the growth of branches of approximately 60^o, whereas 'x'-shaped crosslinks are induced by spectrin or filamin links. This presents the potential to use a range of techniques for guiding *in vitro* actin growth such as micropatterning of growth factors on solid surfaces, although this represents a separate track from computing with live substrates (but still technically represents biological UC).

We propose that the most viable route to computing with *in vivo* vesicle collision circuitry would be to design CFV collisions around common protein conformations: as these are highly conserved and the topology of a cell's actin network may be influenced through guiding patterns of cell growth with environmental sources of stimulation [65, 66], this may be considered as a programmable process.

Loading of vesicles onto microfilaments is a comparatively easy task as binding of myosin-conjugated objects to actin via localised release from a micropipette is a well-observed phenomenon [189]: this only presents the requirement to procure myosin-tagged CFVs, which we propose could be harvested through differential centrifugation of cell lysate [314] or via *in vitro* synthesis of giant unilamellar vesicles in the presence of lyphilised myosin [198, 280].

Should these technical barriers be overcome, we conceive that VCM circuits will be an extremely powerful computing resource, capable not only of amorphous computation but also high information storage density, the retrieval of which poses none of the problems of heat generation that conventional architectures suffer from thanks to the inherent damping of the slime mould system. For completeness, Fig. 5.11 demonstrates a schematic diagram for a VCM half-adder circuit built from one XOR and one AND gate, i.e. one type I and one type III collision: this represents a relatively small technological advance from our current position as there are multiple network topologies based on Arp2/3 branches and spectrin crosslinks that could support its function, hence only collision type and vesicle loading onto the network need be defined. We estimate that a two-gate device such as this may be as small as 100 μm in length (two individual junctions with a realistic spacing of 50 nm [121, 316]) with an operation time of 500–1000 ms, based on an average value of known vesicle transport velocities across this distance [176, 293]. Both of these values are significantly more desirable than any of the previously mentioned biological logical devices, assuming that they could be made reliable.



Input		Output	
X	Y	Sum	Carry
0	0	0	0
0	1	1	0
1	0	1	0
1	1	0	1

FIGURE 5.11: Schematic diagram to demonstrate the construction and operation of a VCM half-adder circuit. If only one vesicle $\langle X \rangle$ or $\langle Y \rangle$ is input, it will pass down the sum XOR path, whereas if both $\langle X, Y \rangle$ are input both vesicles will fuse upon collision, leading to a single signal being present on the carry AND path. Arrow indicates direction of vesicle transport. Truth table is inset where sum represents the $\langle XY \rangle$ path and carry the $\langle X \bigoplus Y \rangle$ path. Path lengths not to scale. Adapted from the author's own work in Ref. [205].

5.4.4.5 Conclusions

Practical VCM circuitry represents the first realisation of *in vivo* collision-based computing and promises far greater speed of operation, informational density and parallelism than any other *Physarum* machine fabricated to date. VCM computing will not emerge without a significant research effort, however.

We have identified an energetic process within the *P. polycephalum* plasmodium which can be characterised in the language of computation, identified the key characteristics of the medium and presented as our recommendations for further study a realistic methodology by which practical VCM circuitry may be implemented. As such we have proposed a framework by which a SMIP device may be fabricated; the designs for cytoskeletal circuitry presented may also support other types of energetic event transfer and processing, such as those detailed in section 5.1.

With regards to commenting on the polymorphism of the SMIP as a UC substrate, VCM circuitry would necessitate entirely novel forms of input and output arising from subtle microscopic manipulation and observation/other means of recording. Possessing the means to assemble universal logic gates theoretically opens the doors to general-purpose computation so promises true polyfunctionality, although it is impossible to comment on a range of uses for such devices at this stage. Finally, the homology between the

cytoskeletons of all eukaryotic cells implies that this technology need not necessarily be restricted to slime mould cells.

Chapter 6

Summary and Conclusions

6.1 Summary and Critical Evaluation of Findings

Let us consider how the thesis:

> Slime mould *Physarum polycephalum* is a polymorphic, polyfunctional unconventional computing substrate[1].

and research questions:

1. Which slime mould morphological and physiological parameters may be utilised as measurable, statistically-repeatable output in a slime mould computing device?
2. How are data and computing tasks represented in a slime mould computing device, how can it be programmed and how is an output perceived and interpreted by the user?
3. To what extent can slime mould computing be used practically and how can the knowledge acquired be applied to the wider field of UC?

that were originally presented in Chapter 2 were addressed by the research documented in Chapters 3, 4 and 5.

[1] For completeness, the antithesis of this statement is that slime mould cannot process multiple input types with its cellular hardware or be put to use in multiple computing applications.

6.1.1 Heterotic Devices (SMHDs)

Initially, a novel class of optically-coupled electrical slime mould NAND gate was presented. The logical gates developed hold little practical value, but demonstrate key design principles for SMHDs, namely that electrical interfacing is suitable for automated recognition of morphological operations and that multisensorial stimulation of the organism via chemical and light sources may be used to provoke behaviour patterns consistent with functionally complete Boolean logic.

Secondly, a basic tactile sensor built upon an FPGA interface was presented. Again, although this device's practical usefulness is limited, it represents a low-cost, adaptable platform upon which future iterations of slime mould devices with demonstrable practical functions could be developed. Consider, for example, a plasmodial tubule network overlying a multi-electrode array, where each electrode is interfaced to an FPGA input pin (the board used in section 3.2 may accept 40 separate inputs) via their own ADC circuits. With suitable programming and hardware adaptations to allow the user to capitalise upon the organism's capacity for polymorphic input, the FPGA can be used to supply optical, thermal or electrical stimulation via LEDs, Peltier elements or impulses directed to certain electrodes, towards instantiating multi-bit operations in plasmodial tubule networks. Periodic stimulation of the organism via the FPGA is, furthermore, a viable route towards entraining plasmodial tubules into producing anticipatory responses interpreted by the interface through electrophysiological measurements. Combined with suitable advances in SMMP operation, we envisage this to be a viable route towards implementing fully-automated slime mould analogue computers with a wide range of functions (as was discussed in Chapter 4).

Thus, the advances detailed in Chapter 3 have indicated suitable forms of input and output in SMHDs as well as the feasibility of producing functional devices built on the principles outlined in Chapters 4 and 5. It is also pertinent to note that the slime mould–computer interface developed is eminently well-suited for use with other excitable cell types: despite the slow progress of experimental biological UC due to the field's inherent multidisciplinarity excluding researchers not possessing skills in both biology and computer science, as was discussed in the introduction, this low-cost, easy-to-use interface represents a step towards greater accessibility in UC.

6.1.2 Morphological Processors (SMMPSs)

It was demonstrated in Chapter 4 that the SMMP is able to represent the shape of a dataset in a manner approximating various image processing operations, when the

user-supplied 'image' is mediated through attractant and repellent gradients. These operations, computation of the convex hull, (to some extent) the concave hull, skeletonization and representation of shapes, demonstrate that the organism's 'toolbox' of image processing functions extends far beyond anything previously demonstrated although they were indicated by Jones's multi-agent model. The use of sensorial fusion in these devices goes some way to demonstrate polymorphism in the substrate but no advances were made in elucidating exactly how each input variety was represented within the organism.

Consequent experiments demonstrating that the organism is able to display anticipatory responses to adverse periodic stimuli showed that the SMMP can be considered to function as an analogue computer complete with integrators (biological feedback mechanisms) and basic memory capabilities (through oscillator coupling) when its output is considered as the spatiotemporal dynamics of a single plasmodial tubule. Again, these results demonstrate a marked increase in functionality over anything previously demonstrated for SMMPs. Although only one variety of input was used in programming these devices (light), significant evidence was advanced to elucidate how data is represented within the organism (as oscillating biomolecular processes) and the processes which constitute computation (reactions which maintain and influence these oscillating processes). Indeed, this latter point highlights an intriguing characteristic of the *P. polycephalum* plasmodium which lends it value as an UC substrate, namely that observation of morphological adaptation also allows one to infer maps of spatial stimuli as well as cytoplasmic flow (and hence related oscillator patterns). This is an embodiment of the concept of spatial propagation of information towards continual monitoring of the computing process, as well as related processes.

Relying on morphological adaptation as a form of output makes device operation extremely slow, with individual operations taking many hours to complete. Even so they are not without practical use: there have been numerous publications [16, 32] demonstrating how SMMP devices have been used for bio-inspired design through computation of space subdivision, thereby indicating that the novel morphological operations may also be oriented in a similar direction. With regards to SMMP analogue computers, the only practical device described was an AND gate implemented via the organism's systems we have characterised as a form of memory, hence it cannot be said with any certainty how viable more complex devices built around this phenomenon may be. That said, the corroboration between the results gathered and Blakey's predictions [60] regarding the arithmetical applications of living coupled oscillator systems indicate that with sufficient development, SMMPs may be capable of computing problems such as factorisation in a more efficient manner than conventional architectures. Although we do not intend to suggest that a SMMP factorising a number over the course of an hour would be

more efficient than a modern computer running a fundamentally inefficient factorising algorithm, and there are furthermore several limitations to the SMMP that were not explored (e.g. maximum number that can be represented through periodic stimuli), further work on this topic would be likely to further our understanding of the computing process in biological substrates in general.

6.1.3 Intracellular Processors (SMIPs)

Guided by a literature review of potential intracellular systems for implementing computation, it was demonstrated through CA modelling that actin cytoskeleton-mediated signalling processes could theoretically support the generation of travelling localisations and interactions therein. These modelling experiments also contributed some evidence towards extant theories concerning how emergent processes within cells are facilitated by the cytoskeleton; of course it is beyond the remit of modelling to ever fully substantiate these hypotheses, but such techniques may still be of use in cases such as this, where the technology to directly measure the phenomena under observation does not exist, in order to guide experimental work.

Further experimentation demonstrated that calcium-filled vesicle (CFV) dynamics on intraplasmodial networks may be characterised as CBC-like computation. Although no prototype SMIPs with demonstrable practical functions were presented, the investigations in Chapter 5 demonstrate a clear basis by which unstructured environmental sensory data is transduced into a finite number of intracellular signal types, many being mediated by the cytoskeleton. This process of information structuring creates unambiguous signals to which the organism will react in a specific, consistent manner. A particularly enthusing characteristic of vesicle collision model (VCM) computation is that it shares functional characteristics with Fredkin and Toffoli's billiard ball model (BBM), hence a functionally complete set of logical gates may be constructed using basic 2-vesicle interaction gates.

As with the SMMPs developed in Chapter 4, we were only able to demonstrate in the experimental portion of Chapter 5 how naturally-occurring processes may be utilised as computing resources without elucidating a method for 'hijacking' them to maximise their usefulness i.e. vesicle collisions on actin highways may be observed and somewhat engineered through directing the macroscopic growth of the organism, but not tightly controlled. Although achieving this level of control over such a system would require a prolonged, concentrated research effort, our findings nevertheless suggest that this is possible. Cytoskeletal information processors are well-suited to miniaturisation, offer excellent data storage density, pose no problems with excess heat production and would

be a practical means of influencing cell dynamics *in vivo*. Cytoskeletal computing architectures are not limited to slime mould models, but the phenomenon of CFV collisions is due to their role being filled by other organelles in different cell types. We recommend that cytoskeletal information processors based on other cell types focus upon the interactions of ionic waves due to this being a phenomenon common to all cell types [155].

6.2 Regarding the Future of Slime Mould Computing, Biological UC and Computer Science

Although slime mould computers are now amongst the most heavily-researched biological UC substrates to date, it must be remembered that *P. polycephalum* was chosen because it is an arguably 'simple' entry-level biological substrate. It is apparent that many of its lauded properties adversely affect its potential for adaptation into demonstrably useful devices: its enormous size, for example, makes slime mould devices ill-suited to miniaturisation, and its obligate unicellularity precludes any form of synergistic interactions with cooperative neighbouring cells.

If, however, slime mould computing research does not fully represent the capabilities of biological substrates in general, this raises the question as to what contribution it makes to the field of biological UC. Can the functions of a SMMP be compared to those of any other cell type? Whilst human neurons possess a similar stellate morphology in certain anatomical locations, their function is known to be heavily dependent on this [212] and hence, it seems unlikely that their full potential could be utilised in a device that was based on neural morphological adaptation (which is currently very difficult to achieve *in vitro* [98]). If we broaden our horizons to the actions of cells outside what has been achieved *in vitro*, however, mammalian embryonic cells can be considered as being capable of extremely complex morphological computation and self-assembly during embryogenesis (cell differentiation, routing of neurons and organs, etc.). Thus we see that this incredible process — which is still only partially understood — may be characterised as the computation of various morphological adaptations in response to environmental stimuli (the actions of neighbouring cells and the uterine environment). Once again we return to the notion that UC is a feasible route towards understanding and possibly even hijacking biological processes; the biomedical applications of reverse-engineered embryonic cell computing are diverse and world-altering.

Anticipation and biological oscillation in general are also features of other biological substrates, implying that the techniques used to implement the SMMP analogue computing devices presented are transferable to other cell types, provided that the oscillating processes in said substrates were suitably elucidated.

The SMIP designs based on transmission of and interactions between energetic signalling events on actin networks are, conversely, applicable to any cell type or even *in vitro* actin networks. It should be noted that most other cell types are unlikely to contain as many CFVs as *P. polycephalum* plasmodia as their function is supplanted by other organelles, but vesicles are a ubiquitous mechanism for carrying a wide range of biomolecules in animals, plants, bacteria and fungi. Furthermore, as discussed in Chapter 5, actin is highly likely to mediate other forms of energetic event that may be far better suited to ease of input and output, such as transmission of ionic waves when in the presence of a suitable buffer, which could be measured with relative ease using patch clamp electrophysiology [189]. Similarly, whilst it would be very difficult to fabricate electrically-coupled logic gates or tactile sensors with other cell varieties, the design principles demonstrated — favouring of electrical interfacing via multi-electrode array, etc. — are highly transferable between other forms of cell, provided they are electrically excitable, as was discussed in Chapter 3. Again, the transfer of knowledge from biology to UC is not unidirectional: many cytoskeletal processes are poorly understood but may be investigated further from a computational perspective.

It would appear, therefore, that although slime mould is an ideal 'entry level' model organism for UC research, the range of practical applications for *Physarum* machines are limited. Primarily, though, we have demonstrated the viability of adapting entire live cells as well as their components into functional UC devices and uncovered design principles for the fabrication of future generations of biological UC devices. We recommend that the choice of cell type in future experimentation in the field should depend on the requirements of the application — e.g. bio-computers with biomedical applications should focus on the use of human cells, biosensors designed to interpret optical input should use photosensitive cells such as algae, etc. — as it is likely that the principles of intracellular computation are similar between a great many cell types.

It is pertinent to briefly address the contributions made during this program of study towards the parent field of computer science. Our results have made it abundantly clear that, although a great many of the processes we have investigated may be characterised in the language of computation, there is significant divergence between biological and conventional computation. As such, we find that our contributions to conventional computing are limited: we have demonstrated the inefficiency of using biological substrates to implement conventional digital logic and accordingly, there is little in our findings

that can inform future developments in silicon architectures. We find, however, that our work on organism-computer interfaces, in which we enabled communication between the two in formats readily recognisable by both, has uncovered engineering and programming principles which could conceivably contribute to the study and development of trans-architectural data operations.

6.3 Conclusions

In conclusion, to address the research questions recapitulated in section 6.1:

1. We have identified that *P. polycephalum* is polymorphic to the extent that it can be given input in a variety of unstructured formats — chemical, optical, tactile etc. — and responds by producing a number of proportional, ordered, statistically-repeatable behaviours as a result of an intracellular computation-like process. We have elucidated the nature of some of these intracellular processes to some degree. These forms of output include membrane potential absolute values/waveforms and macroscopic behaviour patterns. Where it was previously known that slime mould responds to some forms of input such as light and attractive chemical stimuli, we have elaborated further on how these may be employed through novel experimental environments and methods of input application.

2. Unstructured environmental sensory data is transduced into a regular, repeatable format (structuring) through interaction with membrane-bound or cytoplasmic receptors which initiate signalling cascades; a significant number of these processes are mediated by the cytoskeleton. The end-product of these cascades is an alteration in the concentrations of allosteric effector biomolecules which have measurable effects on the cell's behaviour, such as biasing oscillating intracellular processes. Intracellular data are therefore represented by biomolecules, their states and the bioelectrical, biochemical or biomechanical effects. Computation therein represents the continuous process from signal transduction, transmission, interaction with other quantities of data and their interactions with the systems they effect. Output may be observed in a variety of ways, as discussed in the previous point.

3. We have demonstrated that all three categories of device presented may be adapted into computing devices, each of which can be said to fulfil a demonstrable computing function. Although general purpose computation has not been achieved with slime mould devices (as one would expect given the comparative infancy of biological computing technologies), we have demonstrated the fabrication and operation

of both special purpose architectures as well as systems capable of computationally universal logic.

Through these research questions, we have demonstrated the extent to which the *P. polycephalum* plasmodium can be called polymorphic: the multiple formats of input data used in experimental studies are structured via a series of common intracellular chemical reactions; that these reactions are common between input types is evident in the innervation of the same systems, e.g. light and chemical inputs interact with the energy supply and biomechanical oscillators in order to provoke directional migration.

Furthermore, this demonstrates the extent to which the organism can be called a polyfunctional substrate: by illustrating how various cellular systems, whose operation can be characterised in the language of computing, we have shown that the organism is capable of functionally-complete logic, memory and various morphological functions, which can be practically expressed as bitwise operations, sensing, image processing, etc.

Thus our synthesis: the plasmodium of slime mould *P. polycephalum* is polymorphic to the degree that it can accept and process multimodal input, and is only polyfunctional insofar as being able to complete a narrow range of functions, although the substrate is not intrinsically limited to just the functions that were demonstrated during this program of research. Thus, we conclude that slime mould is not ideally suited to general purpose computation and, without significant further development, suffers from the detriments arising from the variability, fragility and time restrictions of biological substrates. We find, however, that it possesses certain intracellular systems (oscillators, cytoskeletal energy dynamics etc.) common to most forms of life, that are well-suited for adaptation into biological UC devices with demonstrable practical purposes due to possessing some clear advantages over extant computing architectures, both conventional and unconventional.

Appendix A

Appendix A: Content Disclaimers

The work presented in this document draw on the following published works produced or co-produced by the author: [34, 164, 201–209]. All such material is fully referenced and reproduced data is included with express permissions of the copyright holders. Any material reproduced from such a publication that was not produced exclusively by the author (i.e. co-authored work) is detailed here.

The author declares no conflict of interest and declares funding from the European Union's Seventh Framework Program (FP7), grant number 316366, 'Physarum Chip'.

Chapter 2

This Chapter draws from the published works [201, 203, 204]. All work was produced by the author.

Chapter 3

3.1

This section draws from the published work [206]. The author gratefully acknowledges the contributions of the co-authors of these articles, which were as follows: Andrew Adamatzky aided with result interpretation.

3.2

This section draws from the published work [209]. The author gratefully acknowledges the contributions of the co-authors of these articles, which were as follows: Antisthenis Tsompanas designed the original digitalization circuit and contributed to FPGA programming. Andrew Adamatzky and Georgios Sirakoulis aided with result interpretation.

Chapter 4

4.1

This section draws from the published work [164]. The author gratefully acknowledges the contributions of the co-authors of this article, which were as follows: Jeff Jones

completed all modelling experiments and aided interpreting the laboratory experiments; Andrew Adamatzky wrote the Processing sketch which the author adapted for isolating and filtering an image by constituent colours.

4.2

This section draws from the published work [208]. The author gratefully acknowledges the contributions of the co-authors of this article, which were as follows: Jeff Jones completed all modelling experiments (data not included); Ella Gale provided guidance with Matlab-based signal processing; Andrew Adamatzky aided in result interpretation and contextualisation.

Chapter 5

5.1

This section draws from the published works [203, 207]. The author gratefully acknowledges the contributions of the co-authors of these articles, which were as follows: Jeff Jones completed all modelling experiments (data not included); Andrew Adamatzky aided with result interpretation and undertook preliminary work with actin computing simulations (data not included).

5.2

This section draws from the published work [202]. The author gratefully acknowledges the contributions of the co-authors of these articles, which were as follows: Andrew Adamatzky wrote the Processing sketch for constructing excitable CA, which was then edited and run by the author.

5.3

This section draws from the published work [34]. The author gratefully acknowledges the contributions of the co-author of this article, which were as follows: Andrew Adamatzky conceived of the idea of actin automata and programmed the CA model.

5.4

This section draws from the published work [205]. The author gratefully acknowledges the contributions of the co-authors of these articles, which were as follows: Andrew Adamatzky aided with result interpretation and advanced designs for the vesicle half-adder circuit.

Appendix B

Appendix B: Supplementary Information

B.1 Cellular Automaton Definition

To paraphrase Ilachinski [150], cellular automata (CA) are the simplest mathematical representation of complex (non-linear and typically composed of a great many constituent entities) systems. Originally conceived of by Von Neumann in efforts to replicate biological phenomena [317], a CA comprises some form of an homogenous grid in which individual cells may take one of a finite number of states. Each cell may interact with others in its immediate neighbourhood according to a set of pre-defined rules in discrete time; rules dictate how each cell's state will change in proceeding timesteps based on the states of its neighbours. There are therefore as many rules per CA are there are configurations of cells within a neighbourhood.

There are a great many variations on these fundamental building blocks based on variations in grid topology, dimensionality, synchronicity etc., but all share these basic characteristics. Despite these deceptively simple mechanics, CA may develop astonishing complexity and have been extensively used over the past 70 years to great success in modelling the dynamics of systems as diverse as particles in supercolliders [297], molecules in ideal gases [144] and deoxyribosenucleic acid base pattern evolution [276].

B.2 Estimation of Plasmodial Energy Consumption

A single plasmodium of approximately 2 cm^2 weighing 0.1 g was described in Ref. [14] as subsisting for 5 days from the energy derived from a single oat flake.

Energy value of 50g of oat flakes (Tesco, UK): 791 kJ
Energy value of 1g of oat flakes: $791 \div 50 = 15.82$ kJ
Mass of 1 oat flake (approx.): 5 mg
Energy value of 1 oat flake: $15.82 \div 200 = 0.08$ kJ
Energy from 1 oat flake over 5 days $= 0.08 \div 120 = 0.0006$ kJ $h^{-1} = 600$ J h^{-1}
Energy consumption in Watts $= 600 \div 3600 = 0.16$ W

B.3 Proximity Graph Definition

A proximity graph is a planar graph in the Euclidean plane wherein two nodes are connected if they are defined by a certain rule to be within a local neighbourhood and hence proximate in some manner [124, 200, 300]. Consider the following canonical examples of proximity graphs:

- **Minimum (Steiner) Spanning Tree (MST)**: vertices are constructed in order that all points are connected with the minimum possible total edge length.
- **Relative Neighbourhood Graph (RNG)**: two points are connected by an edge if no other point is closer to either.
- **Gabriel Graph (GG)**: two points x, y are connected if a circular neighbourhood of diameter (x, y) oriented centrally about each is empty.
- **Delaunay Triangulation (DT)**: for a set of nodes, triangles are constructed between sets of three nodes if its circumcircle (circumscribed circle about each point) contains no other nodes.

These proximity graphs are related in the Toussaint hierarchy (named after proximity graph pioneer Godfried Toussaint):

$$MST \subseteq RNG \subseteq GG \subseteq DT \tag{B.1}$$

The Voronoi diagram is a form of plane tessellation constructed about the dual of their DT, such that each individual point is separated into a separate neighbourhood (Voronoi cells). Another form of proximity graph, the beta-skeleton, conceptually sits in the Toussaint hierarchy between the GG and DT: they are a variety of undirected graph wherein two vertices are connected if their proximity falls within a neighbourood parameter which is set manually. The relevance of the beta-skeleton in this context is that they have been used to model actin network assembly in Ref. [203].

B.4 Emergence Definition

A great many behaviours exhibited by natural systems cannot be explained through description of the individual processes which underlie them: consider, for example, that we cannot define consciousness in terms of the electrical activity of individual neurons; that is to say, a single neuron cannot be said to possess a consciousness (or perhaps, a one-hundred-billionth of a consciousness?). To pose a somewhat more quantitative example, ion fluxes resulting from electrical stumulation of heart cells (cardiomyocyte) which cause it to contract cannot be said to be 'pumping blood', but a great many cells within the heart tissue system are said to pump blood. This is a phenomenon known as 'emergence', the definition of which is more of a philosophical problem than a scientific one as the interpretation of something as emergent is subjective under certain definitions of the term. The Stanford Encyclopaedia of Philosophy states that [222]:

> Emergent entities (properties or substances) arise out of more fundamental entities and yet are novel or irreducible with respect to them.

Under this definition, we see that a great many of characteristics of biological matter with regards to their use in UC may be classed as 'emergent'; for example, self-assembly of biomolecules followed by their intracellular organisation of and the spontaneous generation of (arguably) complex structures. Emergent phenomena are not restricted to biology — indeed, a reductionist viewpoint would be that literally everything bar fundamental quantum phenomena are emergent properties of interactions between elementary particles and the forces that act on them — but it has been argued that biological matter is a substrate far more conducive to the generation of novel, emergent behaviours than, for example, chemical or physical systems [195]. This would appear to make sense from an evolutionary perspective, i.e. some of the most overtly emergent structures are formed from the materials that are best suited for facilitating emergence[1], i.e. self-assembling molecules.

B.5 Glossary of Biological Terms

Anatomical Notation

The following anatomical terms are used with respect to slime mould:

[1]The reader will please note our efforts to curtail the tautology in this statement, whose purpose is simply to use Occam's Razor as a heuristic.

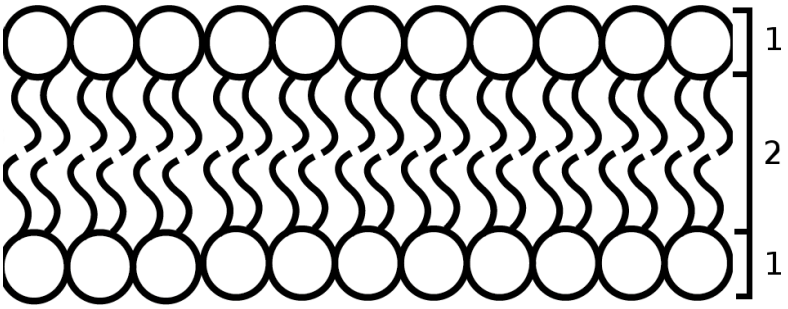


FIGURE B.1: Schematic diagram of a phospholipid membrane in transverse section. '1' denotes hydrophilic (polar) heads and '2' indicates hydrophobic tail regions.

1. **Anterior**: The 'front'; the portion of the organism that advances forwards and usually assumes a fan-shaped morphology.
2. **Posterior**: The 'back'; the parts of the organism which are furthest away from the anterior portions. Usually assumes a tubular morphology.
3. **Caudal**: Meaning 'tail-like', caudal is here used synonymously with 'posterior'.
4. **Margin**: The edge of a physiological layer. E.g. the anterior margin refers to the apex of the organism's anterior portions.

Phospholipid Membrane

A membrane (also known as a phospholipid bilayer, plasma membrane or plasmalemma) (Fig. B.1) is a selectively-permeable boundary between a cell's cytoplasm and extracellular space (in the case of the cell's external membrane) or an organelle (organellar membranes, such as those surrounding the nucleus or mitochondria). Phospholipid membranes are so named because they are formed from two components: a hydrophilic (miscible with water) polarised phosphate-containing 'head' region and two hydrophobic 'tails' formed from fatty acids articulated onto a glycerol backbone. These units form vast sheets which arrange themselves into bilayers tail-to-tail. This prevents the movement of fluid and macromolecules across the membrane; small molecules and some hormones may pass freely through a membrane; trans-membrane proteins may permit the transport of certain larger molecules and ions.

Eukaryote [326]

A cell is said to be eukaryotic if its nucleus (intracellular organelle containing its DNA) is enclosed within its own phospholipid membrane; this includes all animal, plant, fungal and protistic cells. This is contrasted with prokaryotes, such as bacteria, who lack a well-defined nucleus. Eukaryotic cells tend to be larger and be more mophologically complex.

Protist [327]

The protists are a large category of organisms whose designation as such is based on convenient grouping of common attributes, rather than sequential evolutionary heritage (that is to say, polyphyletic rather than monophyletic). Although no universally-accepted formal definition exists to describe the protists, they are informally defined as eukaryotic organisms that are not animals, plants or fungi, who are either unicellular or otherwise multicellular forms of life that do not form tissue systems. Even this rather vague grouping is open to debate, however: algae, for example, were once considered to be plants due to being photosynthetic, but are now widely considered to be protists. This highlights the malleability of our systems for taxonomical classification and hence that we should keep in mind that such groupings only exist insofar as we have created them to ease our understanding of different varieties of organism with common features. Although newer, more systematic classifications have been proposed (most notably, the International Society of Protistologists revised 2012 classification of eukaryotes [36]), these have still note received wide acceptance and hence, the term 'protist' is universally used for these — commonly weird and wonderful — organisms which continue to defy classification.

Ploidy [40]
A cell's 'ploidy' refers to how many sets of chromosomes (structures composed of tightly-compressed DNA) it possesses: it is said to be haploid if it possesses only one set, diploid if it possesses two and polyploidal if it possesses more than two. The relevance of this is that it refers to the stage of a cell's development: vegetative eukaryotic organisms typically have more than one set (most mammals have two, whereas plants may have up to 12 or more), whereas reproductive cells in sexually-reproducing species (gametes) will usually have half the number of chromosomes of the parent. All human cells, for example, are diploid except for spermatozoa and ova, which are haploid: when two gametes of opposite sexes meet and fuse, they form a diploid cell which develops into the vegetative, mature form (i.e. a human foetus).

Spore [328]
Spores are highly resilient life cycle forms possessed by some, but not all, bacteria, fungi and protists. Although not necessarily directly involved in reproduction they usually form an essential part of the organism's life cycle: the spores produced by protists are typically haploid and unicellular as they may eventually transform into gametes when environmental conditions become favourable.

Receptor [244]
In the context of cell biology, a receptor is a protein molecule that exists on the surface of or within a cell whose function is to bind with another protein or small molecule (a

'ligand') and instigate an appropriate response, which is usually to enter a high-energy state and/or change its quaternary structure. This contributes to a change in cellular behaviour: consider, for example, the category of receptor known as an 'ionotropic' receptor that sits on and permeates a cell's membrane. In brain cells (neurons), several varieties of ionotropic receptors exist which each bind to a different ligand, such as gammaaminobutyric acid (GABA) and acetylcholine. When the ligand — which has usually been released from a neighbouring neuron — comes into contact with the receptor, it undergoes a conformational change and begins to permit the passage of certain ions for a limited period of time. The momentary flux of positively-charged ions from the cell's exterior to interior contributes to the generation of the electrical pulses (action potentials) that characterise brain activity.

Second Messengers [245]
Following the activation of a several categories of receptor (e.g. transmembrane G-protein coupled receptors), a cascade of secondary chemical reactions occur which eventually contribute to a change in cell-level behaviour: this is called a second messenger system. Second messengers, such as the cyclic adenosine monophosphate or phosphoinositol systems, are highly-amplifying, energy-consuming chemical processes.

B.6 Excitable System Definition

A generalised excitable system is a simulated two dimensional state space where components may be 'excited', that is enter a functionally different, higher-energy state when perturbed in some way. This state may propagate to neighbouring elements, which gives rise to complex nonlinear system dynamics [147, 306]. These systems are designed to mimic excitable natural systems which obey these rules such as spatially-propagating cell contraction in muscular syncytia (e.g. the heart or bladder) or wave propagation in the oscillating Belousov-Zhabotinsky reaction [30, 329].

B.7 Memristor Definition

Memristors, or 'memory resistors' are a novel circuit element and the last of the quartet of passive circuit elements to be fabricated (as each component forms a link between current, voltage, charge and flux; the memristor links the latter two) [287]. They have been hailed as a potential non-volatile storage medium for their ability to change their resistance to a degree dependent on the last voltage passed through it and maintain this state when the voltage is removed [125].

B.8 Watershed Transformation Definition

The Watershed Transformation is a morphological operation used to segment images that is used in a wide range of application fields including biological imaging, traffic monitoring and materials science [249]. The basic principle of this operation is to consider a greyscale image as a surface with a 3D topography wherein lighter grey tones represent higher surfaces. This 3D surface is then flooded with fluid from its minima and characteristic catchment basins form around the peaks delineated by raised edges. The contours are then marked and output as the 'watershed' of the original image. In complex images, oversegmentation is a frequent problem (as in B.2); this may be remedied by performing an initial edge-finding algorithm to the image to define markers from which the consequent flooding is initiated.

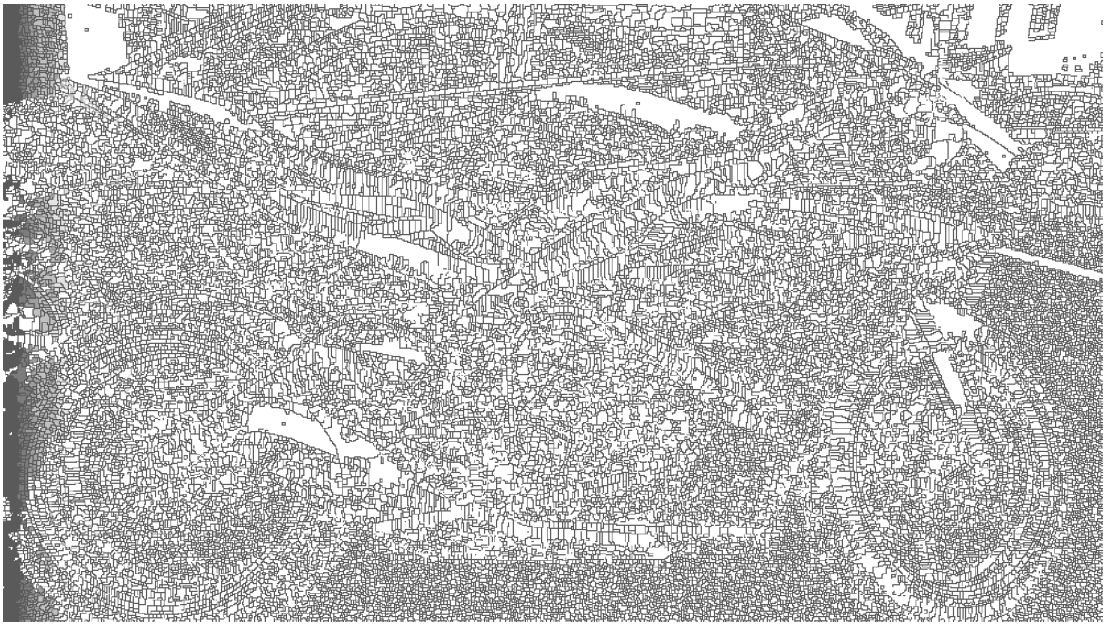
B.9 Collision-based Computing (CBC) Definition

Fredkin and Toffoli's Billiard Ball Model (BBM) of computation [122], which as the name implies is a hypothetical UC device fashioned from billiard balls, is an elegant example of a UC paradigm which demonstrates this singular open-mindedness towards computation. In the BBM, billiard balls of uniform physical attributes representing input data are propelled at set speeds along the gridlines of a regular Cartesian lattice: the machine's output is determined by the presence or absence of balls in predetermined locations after a certain amount of time has elapsed — '1' or logical TRUTH if a ball ends up in location X (potentially sunk down a particular pocket of a billiards table) and *vice versa*. Hence, computation is implemented via conditional routing of balls, which is achieved via engineered ball-to-ball interactions, i.e. elastic ricochets. An example of a billiard ball logical gate is shown in Figure B.3.

At first glance, we find that such a system has virtually no practical applications, would be extremely difficult to implement practically and is a remarkably inefficient route towards making a single logic gate. The BBM is, however, merely an example of a system which implements conservative logic, a non-Boolean logic variety based around the ideas of input-output bijectivity (one-to-one mapping) and reversibility (time-invertibility).

Consider, for example, that in the interaction gate of Fig. B.3 no signals are lost or destroyed, as indicated by the incidence of two output balls when two are input; compare this to the functionality of a conventional AND gate, wherein the conjunction of two input signals leads to only one being output, implying the erasure of the other. Bijectivity implies zero internal energy dissipation (isentropy) in an idealised scenario. In terms of practical circuit design, this confers a conservative logical circuit a superior

(a)



(b)

FIGURE B.2: Demonstration of the watershed transformation. (a) Original image. (b) Watershed transformation of the photograph in [a]. As the original image was complex and no initial markers were defined, the image is oversegmented and hence the morphology of the subject is poorly preserved.

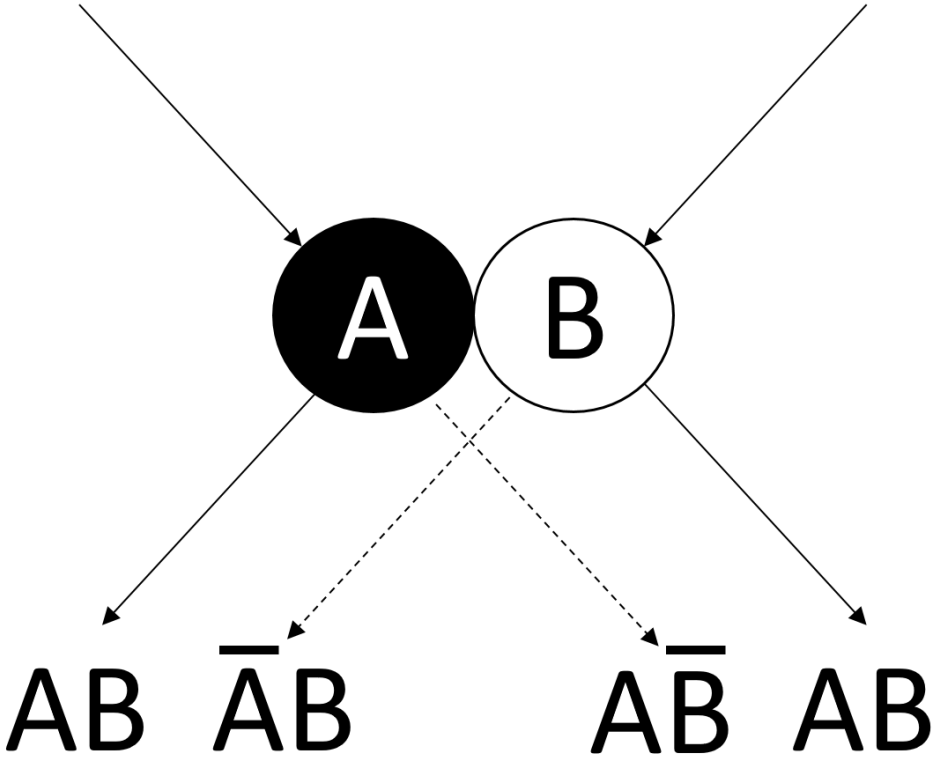


FIGURE B.3: Schematic diagram of a two-input BBM interaction logical gate. When only one input is provided, the ball is free to roll unperturbed along its original trajectory (dashed arrows). When the input configuration is $\langle AB \rangle$, the balls collide and elastically ricochet (solid arrows), re-routing them to a different output configuration representing the conjunction of $\langle A \wedge B \rangle$. The output of the gate may be reconfigured depending on how ball routing is interpreted.

theoretical energy efficiency; aside from the clear advantages of low-energy computing in modern times, this also makes such a system more appropriate for miniaturisation due to the reduction in energy loss as heat (the 'kT barrier') . Another key benefit to a fully-reversible logic is that, as it is a deterministic system, the user is able to make more accurate predictions pertaining to its computing power and resistance to error.

Relatively few laboratory prototypes of BBM-derivative devices (a field of study now known as collision-based computing (CBC)) have been fabricated to date — with perhaps the most notable exception being the successful implementation of Boolean logic via collisions of reactant wavefronts in the oscillating Belousov-Zhabotinsky (BZ) chemical reaction [10, 29, 81] — but the BBM has been thoroughly characterised with various modelling techniques such as cellular automata (CA) [169, 340], which has consequently led to designs for BBM-like computing in quantum computing media such as photons and electrons [58, 142].

B.10 Electronic Supplementary Information

All electronic supplementary information is stored on the attached CD-ROM.

Video 1: Video of excitable CA model run on extracted actin network shown in Fig. 5.2. Excitation is initiated upon mouse click. Black pixels indicate reconstructed actin network, pink are excited cells and blue are refractory cells.

Video 2: Video of excitable CA model run on extracted actin network shown in Fig. 5.3. Excitation is initiated upon mouse click. Black pixels indicate reconstructed actin network, pink are excited cells and blue are refractory cells.

Video 3: Real-time ratiometric confocal microscopy video footage showing calcium-filled vesicles (red) interacting with each other in a live plasmodium. Collisions between vesicles are frequently observed.

Appendix C

Appendix C: Additional Experimental Datasets

C.1 LED Avoidance Observations, Section 3.1

Experiments were performed as per section 3.1.2.1. Experimental observations are presented in Table C.1. The frequency with which each was migrated to was counted; hence, the most avoided colour was the LED variety with the lowest score. The final tally was:

- Green: 0
- Red: 2
- Yellow: 5
- Blue: 9
- No result: 2

The incidence of 'no result' ('X' in Tab. C.1) corresponded to the event where the organism opted to migrate directly upwards onto the lid of the Petri dish, after which it proceeded to colonise one or both of the cardboard dividers. These results were discounted when determining the most-avoided colour.

	Blue	Green	Yellow	Red
Blue	·	B,B,B	B,B,B	B,B,B
Green	·	·	Y,Y,Y	R,X,R
Yellow	·	·	·	Y,Y,X
Red	·	·	·	·

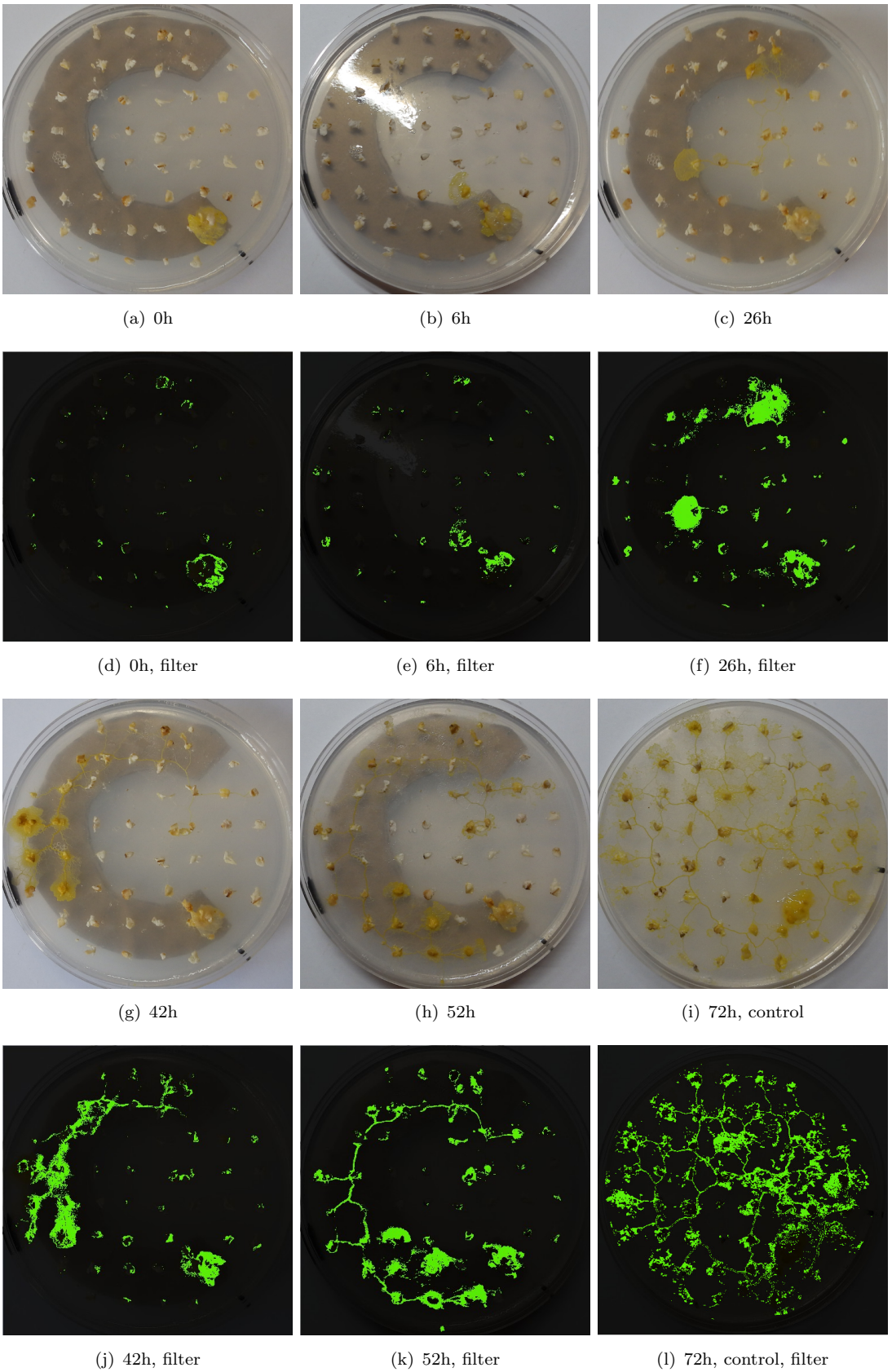
TABLE C.1: Table to show which colour of LED *P. polycephalum* plasmodia migrated to in phototaxis experiments. B = blue, Y = yellow, R = red, G = green, X = no result, · = no experiment performed.

C.2 Morphological Processing Exemplar Datasets, Section 4.1

The following image sets show how slime mould completes the morphological operations outlined in section 4.1. Figure C.1 shows the full single image experiment using a 'C'-shaped mask corresponding to the result shown in Fig. 4.3A. Figure C.2 shows the full double image experiment using a 'C'-shaped mask corresponding to the result shown in Fig. 4.4A. Figure C.3 shows the full double image experiment using a 'H'-shaped mask corresponding to the result in Fig. 4.5A.

C.3 Plasmodial Entrainment by UV Light Exposure Datasets, Section 4.2

This section contains the experimental datasets for the entrainment experiments detailed in section 4.2. Raw time series data is shown in Fig. C.4 and FFTs derived from these are shown in Table C.2.



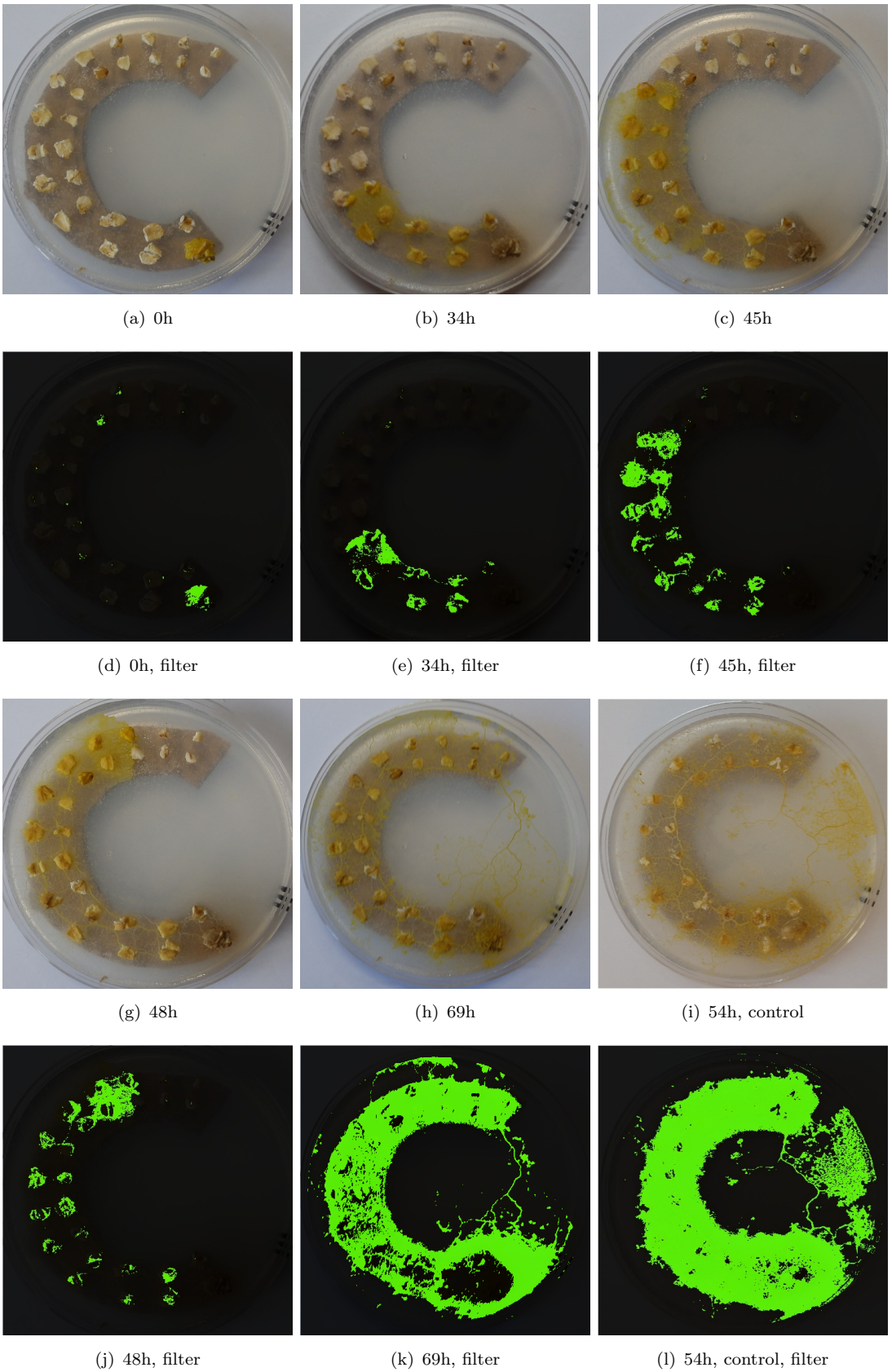
(a) 0h (b) 6h (c) 26h

(d) 0h, filter (e) 6h, filter (f) 26h, filter

(g) 42h (h) 52h (i) 72h, control

(j) 42h, filter (k) 52h, filter (l) 72h, control, filter

FIGURE C.1: Time-lapse photographs of a single image experiment using a 'C'-shaped mask. (a–c,g–i) Experimental photographs; captions indicate time. The organism migrates mainly under the mask and links oats with a minimalistic network of plasmodial tubes. (d–f,j–l) Enhanced images; each corresponds to the photograph immediately above. (i) Control. Elements of figure adapted from author's own work in Ref. [164].



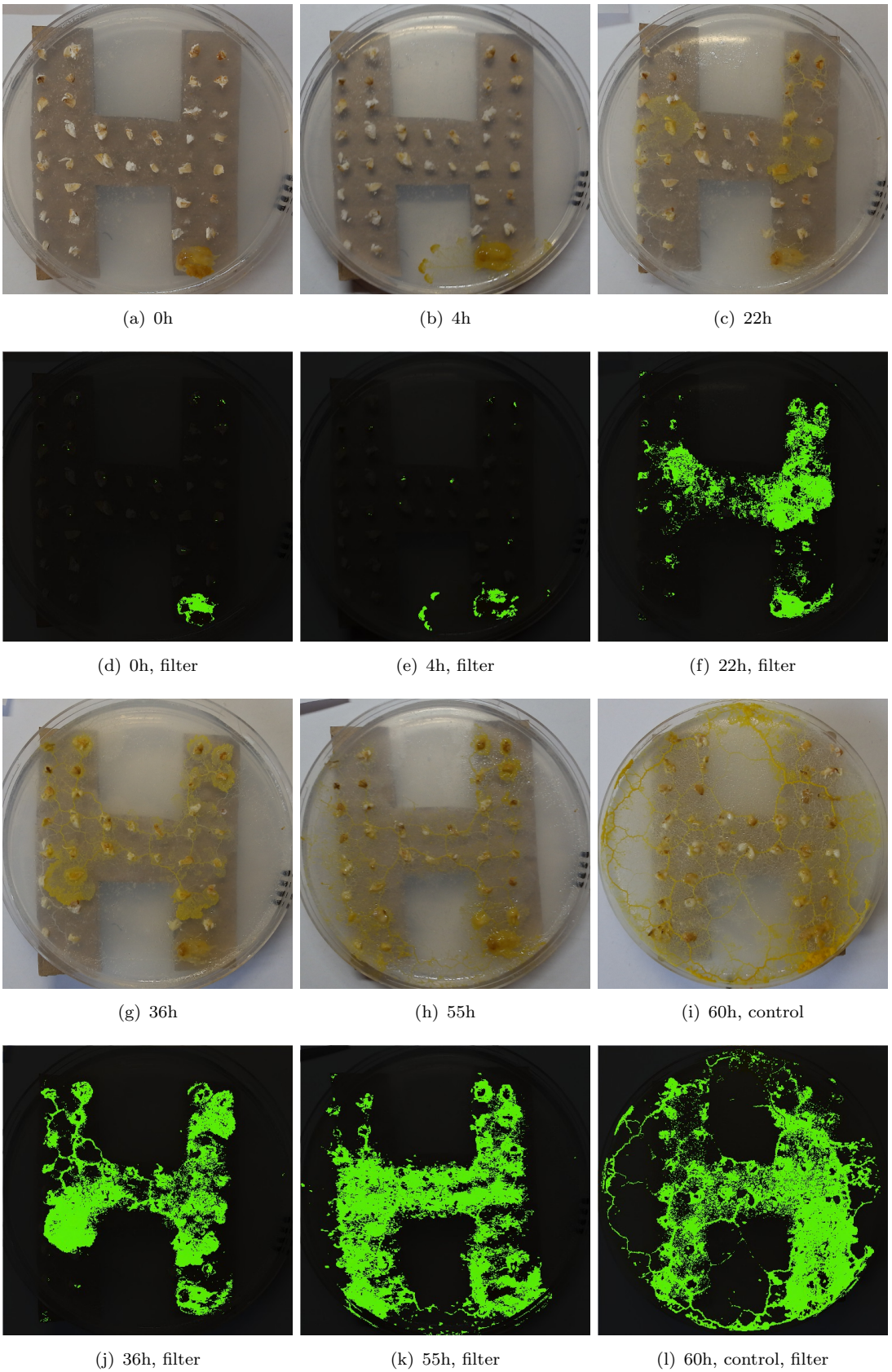
(a) 0h (b) 34h (c) 45h

(d) 0h, filter (e) 34h, filter (f) 45h, filter

(g) 48h (h) 69h (i) 54h, control

(j) 48h, filter (k) 69h, filter (l) 54h, control, filter

FIGURE C.2: Time-lapse photographs of a double image experiment using a 'C'-shaped mask. (a–c,g–i) Experimental photographs; captions indicate time. Initially, the organism colonises the area under the mask. It then opts to link its network across bare agar. (i) Control. Elements of figure adapted from author's own work in Ref. [164].



(a) 0h (b) 4h (c) 22h

(d) 0h, filter (e) 4h, filter (f) 22h, filter

(g) 36h (h) 55h (i) 60h, control

(j) 36h, filter (k) 55h, filter (l) 60h, control, filter

FIGURE C.3: Time-lapse photographs of a double image experiment using a 'H'-shaped mask. (a–c,g–i) Experimental photographs; captions indicate time. The organism colonises the area under the mask first, forming a network redolent of the concave hull of the image. Shortly after, it extends a branch across the gap separating the lower protrusions of the 'H' shape, partially forming the shape's convex hull. (f) Control. Elements of figure adapted from author's own work in Ref. [164].

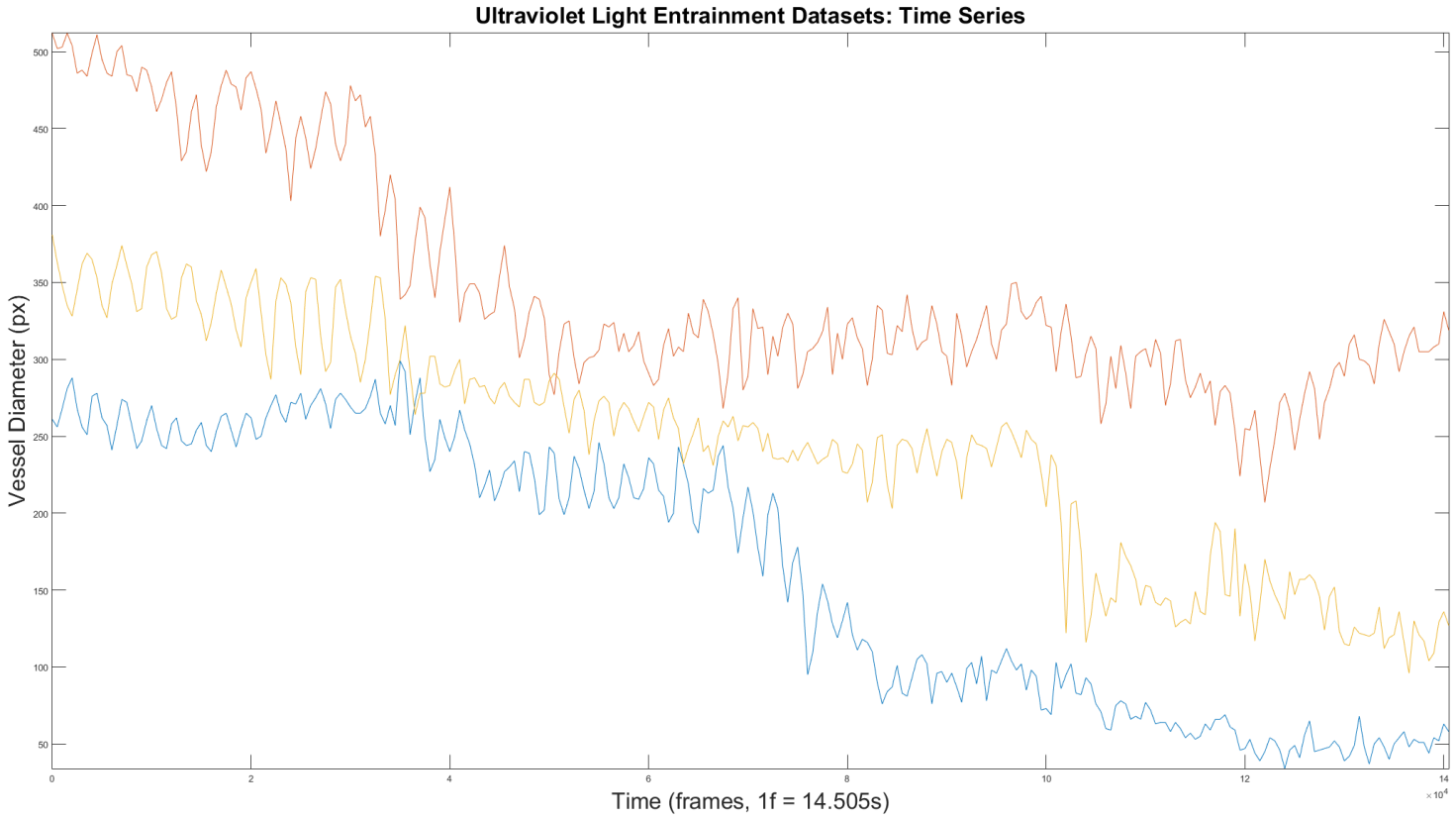


FIGURE C.4: Graph to show changes in vessel diameter over time during UV light entrainment experiments that contribute to the data contained within Table 4.2. Note the downward trend in all datasets. Elements of figure adapted from author's own work in Ref. [208].

TABLE C.2: Table to show fast fourier transformed datasets from UV light entrainment experiments. Data derived from datasets in Fig. C.4 and contribute to Table 4.2. BLF = baseline frequency, see section 4.2 for full definition.

	BLF (Hz)	S1 (Hz)	R2 (Hz)	S2 (Hz)	R3 (Hz)	S3 (Hz)	R4 (Hz)	A (Hz)	DeltaA-R4	DeltaA-BLF drift
Control 1	0.009	0.013	0.014	0.012	0.013	0.015	0.010	0.011	110.00%	11.11%
Control 2	0.008	0.016	0.014	0.013	0.012	0.011	0.013	0.013	100.00%	0.00%
Control 3	0.008	0.017	0.09	0.012	0.011	0.013	0.012	0.013	108.33%	12.50%
Test 1	0.012	0.008	0.011	0.007	0.013	0.013	0.012	0.016	133.33%	33.33%
Test 2	0.010	0.010	0.013	0.012	0.013	0.013	0.014	0.017	121.43%	30.00%
Test 3	0.013	0.012	0.012	0.009	0.013	0.012	0.012	0.017	141.67%	38.46%
High Intensity	0.008	0.010	0.008	0.018	0.008	0.016	0.006	0.031	516.67%	312.50%

List of Figures

List of Tables

Abbreviations

UC	Unconventional Computing
BBM	Billiard Ball Model
DNA	Deoxyribonucleic Acid
BZ	Belousov-Zhabotinsky (reaction)
CA	Cellular Automaton/Automata (pl.)
FPGA	Field-Programmable Gate Array
ADC	Analogue to Digital Converter
ATP	Adenosine Triphosphate
NNA	Non-nutrient Agarose
UV	Ultraviolet (light)
LED	Light-Emitting Diode
SMMP	Slime Mould Morphological Processor
SMIP	Slime Mould Intracellular Processor
SMHD	Slime Mould Hybrid Device
cAMP	Cyclic Adenosine Monophosphate
SSMME	Standard Slime Mould Microscopy Environment
MEA	Multi-electrode Array
ALG	Anticipatory Logic Gate
CBC	Collision-Based Computing
CFV	Calcium-Filled Vesicle
VCM	Vesicle Collision Model

Variables and Units

W	Power	Watts
m	Length	Metre
l	Volume	Litre
s	Time	Seconds
h	Time	Hours
Cd	Luminous Intensity	Candela
Hz	Frequency	Hertz
V	Electrical Potential	Volts
A	Electrical Current	Amperes
J	Energy	Joules

Bibliography

[1] *Molecular cell biology.* W. H. Freeman, New York, USA, fourth edition, 2000.

[2] Nearest neighbours in high-dimensional spaces. In J. Goodman, J. O'Rourke, and P. Indyk, editors, *Handbook of discrete and computational geometry*, chapter 39, pages 877–892. CRC Press, 2004.

[3] K. Å ström and R. Murray. *Feedback Systems: an introduction for scientists and engineers.* Princeton University Press, 2012.

[4] H. Abelson, D. Allen, D. Coore, C. Hanson, E. Rauch, G. Sussman, and R. Weiss. Amorphous computing. *Proceedings of the ACM*, 43(5):74–82, 2000.

[5] H. Abelson, J. Beal, and G. Sussman. Amorphous computing. Technical report, MIT, 2007.

[6] F. Achenbach, U. Achenbach, and D. Kessler. Calcium binding sites in plasmodia of Physarum polycephalum as revealed by the pyroantimonate technique. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*, 32(11):1177–1184, 1984.

[7] A. Adamatzky. Collision-based computing in biopolymers and their automata models. *International Journal of Modern Physics*, 11(7):1321–1346, 2000.

[8] A. Adamatzky. *Computing in nonlinear media and automata collectives.* IOP Publishing, Bristol, UK, 2001.

[9] A. Adamatzky. New media for collision-based computing. In A. Adamatzky, editor, *Collision-based Computing*, chapter 14, pages 411–442. Springer, 2002.

[10] A. Adamatzky. Collision-based computiing in belousov-zhabotinsky medium. *Chaos, Solitons and Fractals*, 21(5):1259–1264, 2004.

[11] A. Adamatzky. Physarum machines: encapsulating reaction-diffusion to compute spanning tree. *Die Naturwissenschaften*, 94(12):975–980, 2007.

[12] A. Adamatzky. Developing proximity graphs by *Physarum polycephalum*: Does the plasmodium follow the Toussaint hierarchy? *Parallel Processing Letters*, 19(105), 2008.

[13] A. Adamatzky. Manipulating substances with Physarum polycephalum. *Materials Science and Engineering: C*, 30(8):1211–1220, 2010.

[14] A. Adamatzky. *Physarum machines: computers from slime mould*. World Scientific Publishing, London, UK, 2010.

[15] A. Adamatzky. On attraction of slime mould physarum polycephalum to plants with sedative properties. *Nature Precedings*, 2011.

[16] A. Adamatzky. *Bioevaluation of world transport networks*. World Scientific Publishing, 2012.

[17] A. Adamatzky. On diversity of configurations generated by excitable cellular automata with dynamical excitation intervals. *International Journal of Modern Physics C*, 23(12), 2012.

[18] A. Adamatzky. Patterns of conductivity in excitable automata with updatable intervals of excitations. *Physical Review E*, 86(5):e056105, 2012.

[19] A. Adamatzky. Routing physarum with repellents. European Physics Journal E, Soft Matter(4):403–410, 2012.

[20] A. Adamatzky. Slime mould computes planar shapes. *International Journal of Bio-Inspired Computation*, 4(3):149–154, 2012.

[21] A. Adamatzky. Physarum wires: self-growing self-repairing smart wires made from slime mould. *Biomedical Engineering Letters*, 3:232–241, 2013.

[22] A. Adamatzky. Slime mould tactile sensor. *Sensors and Actuators B: Chemical*, 188(1):38–44, 2013.

[23] A. Adamatzky. Towards slime mould colour sensor: recognition of colours by *Physarum polycephalum*. *Organic Electronics*, 14(12):3355–3361, 2013.

[24] A. Adamatzky. Slime mould electronic oscillators. *Microelectronic Engineering*, 124:58–65, 2014.

[25] A. Adamatzky, editor. *Atlas of Physarum Computing*. World Scientific, 2015.

[26] A. Adamatzky. *Physical maze solvers. All twelve prototypes implement 1961 Lee algorithm*. 2016. Preprint ahead of publication, available from `http://arxiv.org/abs/1601.04672`.

[27] A. Adamatzky, R. Armstrong, J. Jones, and Y. Gunji. On creativity of slime mould. *International Journal of General Systems*, 32(5):441–457, 2013.

[28] A. Adamatzky and L. Chua. Phenomenology of retained refractoriness: on semi-memristive discrete media. *International Journal of Bifurcation and Chaos*, 22(1):e1230036, 2012.

[29] A. Adamatzky and B. D. L. Costello. Experimental logical gates in a reaction-diffusion medium. *Physical Review E*, 66(4 pt 2):046112, 2002.

[30] A. Adamatzky, B. D. L. Costello, and T. Asai. *Reaction-diffusion Computers*. Elsevier, Amsterdam, The Netherlands, 2005.

[31] A. Adamatzky and J. Jones. Programmable reconfiguration of *Physarum* machines. *Natural Computing*, 9:219–237, 2009.

[32] A. Adamatzky and J. Jones. Road planning with slime mould. if *Physarum* built motorways it would route M6/M74 through newcastle. *International Journal of Bifurfaction and Chaos*, 20(10):3065, 2010.

[33] A. Adamatzky and J. Jones. on Electrical Correlates of Physarum Polycephalum Spatial Activity: Can We See Physarum Machine in the Dark? *Biophysical Reviews and Letters*, 06(01n02):29–57, jun 2011.

[34] A. Adamatzky and R. Mayne. Actin Automata: Phenomenology and Localizations. *International Journal of Bifurcation and Chaos*, 25(2), 2015.

[35] A. Adamatzky and T. Schubert. Slime mold microfluidic logical gates. *Materials Today*, 17(2):86–91, mar 2014.

[36] S. M. Adl, A. G. B. Simpson, C. E. Lane, J. Luke, D. Bass, S. S. Bowser, M. W. Brown, F. Burki, M. Dunthorn, V. Hampl, A. Heiss, M. Hoppenrath, E. Lara, L. le Gall, D. H. Lynn, H. McManus, E. A. D. Mitchell, S. E. Mozley-Stanridge, L. W. Parfrey, J. Pawlowski, S. Rueckert, L. Shadwick, C. L. Schoch, A. Smirnov, and F. W. Spiegel. The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology*, 59(5):429–514, 2012.

[37] L. Adleman. Molecular computation of solutions to combinatorial problems. *Science*, 266(5187):1021–1023, 1994.

[38] P. K. Agarwal and M. Sharir. Applications of a new space-partitioning technique. *Discrete & Computational Geometry*, 9(1):11–38, 1993.

[39] L. Agnez-Lima, J. Melo, A. Silva, A. Oliveira, A. Timoteo, K. Lima-Bessa, G. Martinez, M. Mederios, P. Di Mascio, R. Galhardo, and C. Menck. Dna damage by singlet oxygen and cellular protective mechanisms. *Mutation Research*, 751(1):15–28, 2012.

[40] B. Alberts, D. Bray, K. Hopkin, A. Johnson, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. *Essential Cell Biology*, chapter 6, page 197. Garland Publishing, Abingdon, UK, third edition, 2009.

[41] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. *Molecular Biology of the Cell*. Garland, New York, USA, fourth edition, 2002.

[42] I. Aleksander, editor. *Neural computing architectures: the design of brain-like machines*. MIT, Cambridge, MA, 1989.

[43] C. Alexopoulos. *The Biology of* Physarum *and* Didymium, volume 1 of *Cell Biology: A series of monographs*, chapter 1: Morphology, Taxonomy and Phylogeny, pages 3–20. Academic Press, 1982.

[44] C. Alexopoulos. *The Biology of* Physarum *and* Didymium, volume 1 of *Cell Biology: A series of monographs*, chapter 5: Plasmodial structure and motility. Academic Press, 1982.

[45] M. Alfonseca, A. Ortega, M. De La Cruz, S. R. Hameroff, and R. Lahoz-Beltra. A Model of Quantum-von Neumann Hybrid Cellular Automata: Principles and Simulation of Quantum Coherent Superposition and Decoherence in Cytoskeletal Microtubules. *Quantum Information and Computation*, 15(1-2):22–36, 2015.

[46] K. Alim, G. Amselem, M. P. Brenner, and A. Pringle. Random network peristalsis in Physarum polycephalum organizes fluid flows across an individual. *Proceedings of the National Academy of Sciences*, 110(33):13306–13311, 2013.

[47] R. Alonso-Sanz and A. Adamatzky. Actin automata with memory. *International Journal of Bifurcation and Chaos*, 26(1):1650019, 2016.

[48] M. Aono and Y. Gunji. Resolution of infiniteloop in hyperincursive and nonlocal cellular automata: Introduction to slime mold computing. In *AIP Conference Proceedings*, volume 718, page 177, 2004.

[49] T. Araki, A. Yamamoto, and M. Yamada. Accurate determination of dna content in single cell nuclei stained with hoechst 33258 fluorochrome at high salt concentration. *Histochemistry*, 87(4):331–338, 1987.

[50] D. Avis, D. Bremner, and R. Seidel. How good are convex hull algorithms? *Computational Geometry: Theory and Applications*, 7(5–6):265–301, 1997.

[51] F. Baluska, D. Volkmann, and D. Menzel. Plant synapses: actin-based domains for cell-to-cell communication. *Trends in plant science*, 10(3):106–11, Mar. 2005.

[52] L. O. Baron, R, E. Katz, T. Niazov, and I. Willner. Logic gates and elementary computing by enzymes. *Journal of Physical Chemistry A*, 110:8548–8553, 2006.

[53] I. Barrantes, G. Glockner, S. Meyer, and W. Marwan. Transcriptomic changes arising during light-induced sporulation in Physarum polycephalum. *BMC genomics*, 11:115, 2010.

[54] S. Basten and R. Giles. Functional aspects of primary cilia in signaling, cell cycle and tumorigenesis. *Cilia*, 2(6), 2013.

[55] C. Bates, T. Seshimo, M. Maher, W. Durand, J. Cushen, L. Dean, G. Blachut, C. Ellison, and C. Wilson. Polarity-switching top coats enable orientation of sub10-nm block copolymer domains. *Science*, 338(6108):775–779, 2012.

[56] J. Baumgardner, K. Acker, S. Crowley, W. Deloache, J. Dickson, L. Heard, A. Martens, N. Morton, M. Ritter, A. Shoecraft, J. Treece, M. Unzicker, A. Valencia, M. Waters, A. Campbell, L. Heyer, J. Poet, and T. Eckdahl. Solving a hamiltonian path problem with a bacterial computer. *Journal of Biological Engineering*, 3(11), 2009. doi:10.1186/1754-1611-3-11.

[57] M. Bechman, J. Clark, A. Sebald, and S. Stepney. Unentangling nuclear magnetic resonance computing. In *Unconventional Computing 2007, Bristol, UK, July 2007*, pages 1–19. Luniver Press, 2007.

[58] S. Blair and K. Wagner. *Gated logic with optical solitons*, chapter 12, pages 355–380. Springer, 2002.

[59] J. Blakely. *Chaos synchronization with mismatch-induced lag or anticipation*, chapter 7. Nova Science Publishers, New York, USA, 2007.

[60] E. Blakey. Towards non-quantum implementations of shor's factorisation algorithm. *International Journal of Unconventional Computing*, 10(5–6):339–352, 2014.

[61] H. Blum. A Transformation for Extracting New Descriptors of Shape. In W. Wathen-Dunn, editor, *Models for the Perception of Speech and Visual Form*, pages 362–380. MIT Press, Cambridge, 1967.

[62] L. Bull. On model-based evolutionary computation. *Soft Computing*, 3(2):76–82, 1999.

[63] L. Bull, J. Holley, B. De Lacy Costello, and A. Adamatzky. Toward turing's a-type unorganised machines in an unconventional substrate: a dynamic representation in compartmentalised excitable chemical media. *Computing Nature*, 7:185–199, 2013.

[64] L. Bull and I. Urokov. Towards neuronal computing: Simple creation of two logic functions in 3d cell cultures using multi-electrode arrays. *Unconventional Computing*, 4(2):143–154, 2008.

[65] O. Campàs, L. Mahadevan, and J. F. Joanny. Actin network growth under load. *Biophysical Journal*, 102(5):1049–1058, 2012.

[66] A. Carlsson. Growth of branched actin networks against obstacles. *Biophysical Journal*, 81(4):1907–1923, 2001.

[67] C. Carpenter. Actin cytoskeleton and cell signalling. *Critical Care Medicine*, 28(4):94–99, 2000.

[68] M. Christen, H. Kulasekara, B. Christen, B. Kulasekara, L. Hoffman, and S. Miller. Asymmetrical distribution of the second messenger c-di-gmp upon bacterial cell division. *Science*, 328(5983):1295–1297, 2010.

[69] G. Church, Y. Gao, and S. Kosuri. Next-generation digital information storage in dna. *Science*, 337(6102):1628, 2012.

[70] M. Cieślawska and A. Grebecki. *Current research on* Physarum, volume 120, chapter Synchronal pulsation in plasmodia of *Physarum polycephalum*, pages 167–170. 1979.

[71] CIRCLE Project. Circle project: About page. Available online at `http://fet-circle.eu/index.php/about-2/`. [Accessed 13 Apr 2016].

[72] M. Ciszak, O. Calvo, C. Masoller, C. R. Mirasso, and R. Toral. Anticipating the response of excitable systems driven by random forcing. *Phys. Rev. Lett.*, 90:204102, May 2003.

[73] D. Clarke and L. Sokoloff. *Basic neurochemistry: molecular, cellular, and medical aspects*, chapter Circulation and energy metabolism of the brain, pages 637–669. Lippincott-Raven, Philadelphia, 6 edition, 1999.

[74] M. Clynes and N. Kline. Cyborgs and space. *Astronautics*, (September):26–27, 74–75, 1960.

[75] P. Cockshott, L. Mackenzie, and G. Michaelson. Non-classical computing: Feasible versus infeasible. In *Proceedings of the 2010 ACM-BCS Visions of Computer Science Conference*, ACM-BCS '10, pages 4:1–4:10, Swinton, UK, UK, 2010. British Computer Society.

[76] J. Collier. Simulating autonomous anticipation: The importance of Dubois' conjecture. *BioSystems*, 91(2):346–354, 2008.

[77] M. Conrad. Functional significance of biological variability. *Bulliten of Mathematical Biology*, 39(2):139–156, 1977.

[78] G. Cooper. *Structure and organization of actin filaments*. Sinauer Associates, 2000. Available from `http://www.ncbi.nlm.nih.gov/books/NBK9908/`.

[79] D. Coore. Introduction to amorphous computing. In J.-P. Bantre, P. Fradet, J.-L. Giavitto, and O. Michel, editors, *Unconventional Programming Paradigms*, volume 3566 of *Lecture Notes in Computer Science*, pages 99–109. Springer Berlin Heidelberg, 2005.

[80] I. B. M. Corporation. Watson supercomputer press page. `http://www.ibm.com/smarterplanet/us/en/ibmwatson/`, 2014. Accessed: 2014-12-23.

[81] B. D. L. Costello. Constructive chemical processors — experimental evidence that shows that this class of programmable pattern forming reactions exist and the edge of a highly non-linear region. *International Journal of Bifurcation and Chaos*, 13:1561–1564, 2003.

[82] T. Craddock, J. Tuszyński, and S. Hameroff. Cytoskeletal signalling: is memory encoded in microtubule lattices by camkii phosphorylation? *PLoS Computational Biology*, 8(3), 2012.

[83] R. Cummings. If it is methylated it must be tectonic. *PNAS*, 111(22):9669–9670, 2014.

[84] H. Cuntz, F. Forstner, A. Borst, and M. Häusser. One rule to grow them all: a general theory of neuronal branching and its practical application. *PLoS Computational Biology*, 6(8), 2010.

[85] R. Daniel, J. Rubens, R. Sarpeshkar, and T. Lu. Synthetic analog computation in living cells. *Nature*, 497:619–623, 2013.

[86] C. Davia. chapter 7: Life, catalysis and excitable media: a dynamic systems approach to metabolism and cognition. Springer Berlin Heidelberg, New York, USA, 2006.

[87] A. Davydov. Solitons and energy transfer along protein molecules. *The Journal of Theoretical Biology*, 66:379–387, 1977.

[88] R. Dawkins. *The Greatest Show on Earth: The Evidence for Evolution.* Bantam Press, 2009.

[89] M. De Berg, O. Cheong, M. Van Kreveld, and M. Overmars. *Computational Geometry: Algorithms and Applications.* Springer, third edition, 2008.

[90] B. P. J. de Lacy Costello and A. I. Adamatzky. Assessing the chemotaxis behavior of Physarum polycephalum to a range of simple volatile organic chemicals. *Communicative & integrative biology*, 6(5):e25030, sep 2013.

[91] T. De Poian, A dna El-Bacha and M. Luz. Nutrient utilization in humans: Metabolism pathways. *Nature Education*, 3(9), 2010.

[92] V. Devi and E. Guttes. Macromolecular syntheses and mitosis in uv-irradiated plasmodia of *Physarum polycephalum*. *Radiation Research*, 51:410–430, 1972.

[93] M. W. Dictionary. Physarum definition. Available from `www.merriam-webster.com/dictionary/physarum`, 2015.

[94] M. Dietrich. Explaining the pulse of protoplasm: The search for molecular mechanisms of protoplasmic streaming. *Journal of Integrative Plant Biology*, 57(1):14–22, 2015.

[95] A. Dimonte, A. Cifarelli, T. Berzina, V. Chiesi, P. Ferro, T. Besagni, F. Albertini, A. Adamatzky, and V. Erokhin. Magnetic nanoparticles-loaded physarum polycephalum: directed growth and particles distribution. *Interdisciplinary sciences: Computational Life Sciences*, 6:1–9, 2014.

[96] A. Dimonte, F. Fermi, and V. Erokhin. Spectral imaging method for studying *Physarum polycephalum* growth on polyaniline surface. *Materials Science and Engineering: C*, 53:11–14, 2015.

[97] D. Drachman. Do we have brain to spare? *Neurology*, 64(12):2004–2005, 2005.

[98] D. Drivastava, K. Woolfrey, and P. Penzes. Analysis of dendritic spine morphology in cultured cns neurons. *Journal of Visualised Experiments*, 53(2794), 2011.

[99] D. Dubois. *Mathematical foundations of discrete and functional systems with strong and weak anticipations*, volume 2684 of *Lecture Notes in Artificial Intelligence*, pages 110–132. Springer, 2003.

[100] D. M. Dubois. Generation of fractals from incursive automata, digital diffusion and wave equation systems. *BioSystems*, 43(2):97–114, 1997.

[101] D. M. Dubois. Introduction to computing anticipatory systems. *International Journal of Computing Anticipatory Systems*, 2:3–14, 1998.

[102] M. Duckham, L. Kulik, M. Worboys, and A. Galton. Efficient generation of simple polygons for characterizing the shape of a set of points in the plane. *Pattern Recognition*, 41(10):3224–3236, 2008.

[103] P. Dufort and C. Lumsden. Cellular automaton model of the actin cytoskeleton. *Cell Motility and the Cytoskeleton*, 25(1):87–104, 1993.

[104] A. Dussutour, T. Latty, M. Beekman, and S. J. Simpson. Amoeboid organism solves complex nutritional challenges. *Proceedings of the National Academy of Sciences of the United States of America*, 107(10):4607–4611, 2010.

[105] P. Dutta, S. Khastgir, and A. Roy. Steiner trees and spanning trees in six-pin soap films. *American Journal of Physics*, 78(215):10.1119/1.3247982, 2010.

[106] D. Egelman and P. Montague. Computational properties of peri-dendritic calcium fluctuations. *The Journal of Neuroscience*, 18(21):8580–8589, 1998.

[107] J. Elgeti and G. Gompper. Emergence of metachronal waves in cilia arrays. *Proceedings of the National Academy of Sciences of the United States of America*, 110(12):4470–5, 2013.

[108] P. H. England. *Fundamental Techniques in Cell Culture*. European Collection of Cell Cultures, second edition, 2015.

[109] U. Erra, B. Frola, V. Scarano, and I. Couzin. An efficient gpu implementation for large scale individual-based simulation of collective behaviour. In *2009 International Workshop on High Performance Computational Systems Biology*, pages 51–58, 2009.

[110] S. Ettienne-Manneville. From signaling pathways to microtubule dynamics: the key players. *Currrent Opinions in Cell Biology*, 22(1):104–111, 2010.

[111] T. Fawcett and A. Higginson. Heavy use of equations impedes communication among biologists. *PNAS*, 109(29):11735–11739, 2012.

[112] G. Feng, Y. Lu, L. Hao, F. Zhang, and G. Long. Experimental simulation of quantum tunneling in small systems. *Nature Scientific Reports*, 3(2232), 2013. DOI:10.1038/srep02232.

[113] C. Fernando, G. Kampis, and E. Szathmáry. Evolvability of natural and artificial systems. volume 7 of *Proceedings of the 2nd European Future Technologies Conference and Exhibition*, pages 73–76, 2011.

[114] J. Fingerle and D. Gradmann. Electrical properties of the plasma membrane of microplasmodia of *Physarum polycephalum*. *Jorual of Membrane Biology*, 68:67–77, 1982.

[115] A. M. Fiore-Donno, S. I. Nikolaev, M. Nelson, J. Pawlowski, T. Cavalier-Smith, and S. L. Baldauf. Deep Phylogeny and Evolution of Slime Moulds (Mycetozoa). *Protist*, 161(1):55–70, 2010.

[116] M. Fischetti. Computers verses brains. *Scientific American*, November 2011 Issue, 2011.

[117] F. Fleischer, R. Ananthakrishnan, S. Eckel, H. Schmidt, J. Käs, T. Svitkina, V. Schmidt, and M. Beil. Actin network architecture and elasticity in lamellipodia of melanoma cells. *New Journal of Physics*, 9(420), 2007.

[118] G. Forgacs, S. H. Yook, P. Janmey, H. Jeong, and C. Burd. Role of the cytoskeleton in signalling networks. *The Journal of Cell Science*, 117(3):2769–2775, 2004.

[119] K. Forinash, M. Peyrard, and B. Malomed. Interaction of discrete breathers in impurity molecules. *Physical Review E*, 49:3400–3411, 1994.

[120] A. Francesconi. Morphological operators plugin for gimp. Open source software, available from `https://github.com/alessandrofrancesconi/gimp-plugin-morphop/releases/tag/v1.0`, 2013. [Accessed 07 Feb 2016].

[121] C. M. Franz and D. J. Müller. Analyzing focal adhesion structure by atomic force microscopy. *Journal of cell science*, 118(Pt 22):5315–5323, 2005.

[122] E. Fredkin and T. Toffoli. Conservative logic. *International Journal of Theoretical Physics*, 21(3–4):219–253, 1982.

[123] R. Füchslin, A. Dzyakanchuk, D. Flumini, H. Hauser, K. Hunt, R. Luchsinger, B. Reller, S. Scheidegger, and R. Walker. Morphological computation and morphological control: steps towards a formal theory and applications. *Artificial Life*, 19:9–34, 2013.

[124] K. Gabriel and R. Sokal. A new statistical approach to geographic variation analysis. *Systematic Zoology*, 18:259–278, 1969.

[125] E. Gale. Tio2-based memristors and reram: materials, mechanisms and models (a review). *Semiconductor Science and Technology*, 29(10):104004, 2014.

[126] E. Gale, A. Adamatzky, and B. D. L. Costello. Slime mould memristors. *Bionanoscience*, 5(1):1–8, 2013.

[127] R. Geraghty, A. Capes-Davis, J. Davis, J. Downward, R. Freshney, I. Knezevic, R. Lovell-Badge, J. Masters, J. Meredith, G. Stacey, P. Thraves, and M. Vias. Guidelines for the use of cell lines in biomedical research. *British Journal of Cancer*, 111:1021–1046, 2014.

[128] M. Gerhardt, H. Schuster, and J. Tyson. A cellular automaton model of excitable media: Ii. curvature, dispersion, rotating waves and meandering waves. *Physica D*, 46:392–415, 1990.

[129] E. Goodman. Axenic culture of myxamoebae of the myxomycete *Physarum polycephalum*. *Journal of Bacteriology*, 111:242–247, 1972.

[130] K. Gotoh and K. Kuroda. Motive force of cytoplasmic streaming during plasmodial mitosis of *Physarum polycephalum*. *Cell Motility*, 2(2):173–181, 2005.

[131] A. Grebecki and M. Cieślawska. Plasmodium of *Physarum polycephalum* as a synchronous contractile system. *Cytobiologie*, 17:335–342, 1978.

[132] J. Greenberg and S. Hastings. Spatial patterns for discrete models of diffusion in excitable media. *SIAM Journal of Applied Mathematics*, 54(3):515–523, 1978.

[133] Y. Gruenhaum and R. Foisner. Lamins: Nuclear intermediate filament proteins with fundamental functions in nuclear mechanics and genome regulation. *Annual Review of Biochemistry*, 84:131–164, 2015.

[134] D. Grünbaum, D. Eyre, and A. Fogelson. Functional Geometry of Ciliated Tentacular Arrays in Active. *The Journal of Experimental Biology*, 2589:2575–2589, 1998.

[135] S. Gueron, K. Levit-Guerevich, N. Liron, and J. Blum. Cilia internal mechanism and metachronal coordination as the result ofhydrodynamicalcoupling. *PNAS*, 94(12):6001–6006, 1997.

[136] Y. Gunji and A. Adamatzky. Robust soldier crab ball gate. *Complex Systems*, 20(2):93–104, 2011.

[137] S. Hameroff. *Ultimate Computing: biomolecular consciousness and nanotechnology*. Elsevier, Amsterdam, Holland, first edition, 1987.

[138] S. Hameroff and R. Penrose. Consciousness in the universe: a review of the 'orch or' theory. *Physics of Life Reviews*, 11(1):39–78, 2014.

[139] M. Hato, T. Ueda, K. Kurihara, and Y. Kobatake. Phototaxis in the true slime mold *Physarum polycephalum*. *Cell Structure and Function*, 1:269–278, 1976.

[140] S. Hato. *Integral Biomathics*, chapter Galvanotaxis of the plasmodium of *Physarum polycephalum*, pages 57–62. Springer, 2012.

[141] H. Hauser, A. J. Ijspeert, R. M. Füchslin, R. Pfeifer, and W. Maass. Towards a theoretical foundation for morphological computation with compliant bodies. *Biological cybernetics*, 105:355–370, jan 2011.

[142] S. Hayashi and O. Takahide. Collision-based compuuting using single-electron circuits. *Japanese Journal of Applied Physics*, 51(6S), 2012.

[143] S. Haykin. *Neural networks: a comprehensive foundation*. IEEE, New York, 1999.

[144] M. Hénon. *On the relation between lattice gases and cellular automata*, chapter 6, pages 160–161. World Scientific, 1989.

[145] H. Hentschel, A. Fine, and C. Pencea. Biological computing with diffusion and excitable calcium stores. *Mathematical Biosciences and Engineering*, 1(1):147–159, 2004.

[146] A. Hodgkin, A. Huxley, and B. Katz. Measurement of current-voltage relations in the membrane of the giant axon of loligo. *Journal of Physiology*, 116(4):424–448, 1952.

[147] A. Holden, M. Markus, and H. Othmer. *Nonlinear wave processes in excitable media*. Springer, Leeds, UK, 1989.

[148] P. Iglesias and P. Devreotes. Biased excitable networks: how cells direct motion in response to gradients. *Current Opinions in Cell Biology*, 24(2):245–253, 2012.

[149] M. Ikeda, T. Tanida, T. Yoshii, K. Kurotani, S. Onogi, K. Urayama, and I. Hamachi. Installing logic-gate responses to a variety of biological substances in supramolecular hydrogelenzyme hybrids. *Nature Chemistry*, 6:511–518, 2014.

[150] A. Ilachinski. *Cellular Automata: A Discrete Automata*. World Scientific, NJ, USA, 2001.

[151] A. Imamoğlu, D. Awschalom, G. Burkard, D. DiVincenzo, D. Loss, M. Sherwin, and A. Small. Quantum information processing using quantum dot spins and cavity-qed. *Physical Review Letters*, 83(20):4204–4207, 1999.

[152] Intel. Pentium p5 microprocessor: product information sheet. Available from `http://datasheets.chipdb.org/Intel/x86/Pentium/24159502.pdf`, 1993. [Accessed 04 Jan 2016].

[153] Intel. Intel 4th generation i7 cpu product brochure. Available from `http://ark.intel.com/products/75131/Intel-Core-i7-4900MQ-Processor-8M-Cache-up-to-3_80-GHz`, 2014. [Accessed 18 Dec 2015].

[154] S. Istrain, S. De-Lean, and E. Davidson. The regulatory genome and the computer. *Developmental Biology*, 310:187–195, 2007.

[155] P. Janmey. The cytoskeleton and cell signalling: component localization and mechanical coupling. *Physiological Reviews*, 78(3):763–781, 1998.

[156] J. Jeter, I. Cameron, N. Smith, W. Steffens, and J. Wille. Cell cycle fluctuations in concentration of various elements in cytoplasm and in nucleus/chromatin of *Physarum polycephalum*. *Journal of Cell Biology*, 35(137):47–62, 1982.

[157] J. Jones. The emergence of dynamical evolution of complex transport networks from simple low-level behaviours. *International Journal of Unconventional Computing*, 6:125–144, 2010.

[158] J. Jones. *From pattern formation to material computation*. Springer, 2015.

[159] J. Jones. *Towards lateral inhibition and collective perception in unorganised non-neural systems*, pages 103–122. 2015.

[160] J. Jones and A. Adamatzky. Towards physarum binary adders. *Biosystems*, 101(1):51–58, 2010.

[161] J. Jones and A. Adamatzky. Slime mould inspired generalised Voronoi diagrams with repulsive fields. *International Journal of Bifurfaction and Chaos*, 2013.

[162] J. Jones and A. Adamatzky. Computation of the travelling salesman problem by a shrinking blob. *Natural Computing*, 13(1):1–16, 2014.

[163] J. Jones and A. Adamatzky. Material approximation of data smoothing and spline curves inspired by slime mould. *Bioinspiration and Biomimetics*, 9(3):036013, 2014.

[164] J. Jones, R. Mayne, and A. Adamatzky. Representation of shape mediated by environmental stimuli in Physarum polycephalum and a multi-agent model. *International Journal of Parallel, Emergent and Distributed Systems*, 2015.

[165] J. Jones, J. Whiting, and A. Adamatzky. Quantitative transformation for implementation of adder circuits in physical systems. *Biosystems*, 134:16–23, 2015.

[166] N. Kamiya. The rate of protoplasmic flow in the myxomycete plasmodium. *Cytologia*, 15(3–4):183–193, 1950.

[167] N. Kamiya. Protoplasmic streaming. *Protoplasmatologica*, 8(3a):1–99, 1959.

[168] N. Kamiya and S. Abe. Bioelectric phenomena in the myxomycete plasmodium and their relation to protoplasmimc flow. *Journal of Colloid Science*, 5(149–163), 1950.

[169] J. Kari. Structure of reversible cellular automata. In *Lecture Notes In Computer Science*, volume 5715, pages 6–6. 8th International Conference on Unconventional Computation, Springer, 2009.

[170] T. Kato and Y. Tomomura. $Ca2^{+}$-sensitivity of actomyosin ATPase purified from *Physarum polycephalum*. *Journal of Biochemistry*, 77(6):1127–1134, 1975.

[171] S. Kauffman. Measuring a mitotic oscillator: the arc discontinuity. *Bulliten of Mathematical Biology*, 36(2):171–182, 1974.

[172] H. Kawamichi, Y. Zhang, M. Hino, A. Nakamura, H. Tanaka, and L. Farkas. Calcium inhibition of *Physarum* myosin as examined by the recombinant heavy mero-myosin. *Advances in Experimental Medicine and Biology*, 592:265–272, 2007.

[173] H. W. Keller and S. E. Everhart. Importance of Myxomycetes in Biological Research and Teaching. *Fungi*, 3:13–27, 2010.

[174] V. Kendon, A. Sebald, and S. Stepney. Heterotic computing: past, present and future. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, 373(2046), 2015.

[175] M. Khan and S. Yousuf. A cardiac electrical activity model based on a cellular automata system in comparison with neural network model. *Pakistan Journal of Pharmaceutical Sciences*, 29(2):579–584, 2016.

[176] K. Kikushima, S. Kita, and H. Higuchi. A non-invasive imaging for the in vivo tracking of high-speed vesicle transport in mouse neutrophils. *Scientific reports*, 3:1913, 2013.

[177] U. Kishimoto. Rhthmicity in the protoplasmic streaming of slime mold *Physarum polycephalum*. *Journal of General Physiology*, 41:1205–1224, 1958.

[178] D. Knuth. Axioms and hulls. In *Lecture Notes in Computer Science*, pages 1–98. Springer, 1992.

[179] K. Kohama. Inhibitory mode for ca2$^+$ regulation. *Trends in Pharmacological Sciences*, 11:433–435, 1990.

[180] A. Kolmogorov and V. Uspensky. On the definition of an algorithm [in russian]. *Uspekhi Matematicheskikh Nauk*, 13(4):3–28, 1958.

[181] I. Kuon and J. Rose. Measuring the gap between fpgas and asics. *IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems*, 26(2):203–215, Feb 2007.

[182] R. Kuroda, S. Hatano, Y. Hiramoto, and H. Kuroda. Change of cytosolic ca-ion concentration in the contraction and relaxation cycle of physarum microplasmodia. In M. Tazawa, editor, *Cell Dynamics*, volume 1 of *Protoplasma*, pages 72–80. Springer Vienna, 1989.

[183] R. Kuroda and H. Kuroda. Calcium accumulation in vacuoles of Physarum polycephalum following starvation. *Journal of cell science*, 44:75–85, aug 1980.

[184] R. Ladner, N. Lynch, and A. Selman. A comparison of polynomial time reducibilities. *Theoretical Computer Science*, 1:103–123, 1975.

[185] R. Lahoz-Beltra, S. Hameroff, and J. Dayhoff. Cytoskeletal logic: a model for molecular computation via boolean operations in microtubules and microtubule-associated proteins. *Biosystems*, 29(1):1–23, 1993.

[186] E. Lander, L. Linton, B. Birren, C. Nusbaum, and *et al.* Initial sequencing and analysis of the human genome. *Nature*, 409(6822):890–921, 2001.

[187] A. Lee, C. Yau, M. Giles, A. Doucet, and C. Holmes. On the utility of graphics cards to perform massively parallel simulation of advanced monte carlo methods. *Journal of Computational and Graphical Statistics*, 19(4):769–789, 2010.

[188] J. Lee, S. Shin, T. Park, and B. Zhang. Solving travelling salesman problems with dna molecules encoding numerical values. *Biosystems*, 78(1–3):39–47, 2004.

[189] E. C. Lin and H. F. Cantiello. A Novel Method to Study the Electrodynamic Behavior of Actin Filaments. Evidence for Cable-like Properties of Actin. *Biophysical Journal*, 65(December 1992), 1993.

[190] Q. Liu, L. Wang, A. Frutos, A. Condon, R. Corn, and L. Smith. Dna computing on surfaces. *Nature*, 403:175–179, 2000.

[191] M. Lungarella and O. Sporns. Information self-structuring: key principle for learning and development. In *Proceedings of 2005 4th IEEE International Conference on Development and Learning*, pages 24–30. IEEE Computational Intelligence Society, Osaka, 2005.

[192] M. Lungarella and O. Sporns. Mapping information flow in sensorimotor networks. *PLoS Computational Biology*, 2(10), 2006.

[193] A. Maniotis, C. Chen, and D. Ingber. Demonstration of mechanical connections between integrins, cytoskeletal filaments and nucleoplasm that stabilise nuclear structure. *PNAS*, 94(3):849–854, 1997.

[194] V. Mansinghka. *Natively Probabilistic Computation.* PhD thesis, Massachusetts Institute of Technology, 2009.

[195] C. Mao. The emergence of complexity: lessons from dna. *PLoS Biology*, 2004.

[196] N. Margolus. *Collision-based Computing*, chapter Universal cellular automata based on the collisions of soft spheres, pages 107–134. Springer, London, UK, 2002.

[197] N. Margolus. Physics-like models of computation. In A. Adamatzky, editor, *Collision-based computing*, chapter 4, pages 83–106. Springer, 2002.

[198] S. Matosevic and B. Peagel. Stepwise synthesis of giant unilamellar vesicles on a microfluidic assembly line. *Journal of the American Chemical Society*, 133(9):2798–2800, 2011.

[199] L. Matthews. Calcium ion regulation in caffiene derived microplasmodia of *Physarum polycephalum*. *Journal of Cell Biology*, 72:502–506, 1977.

[200] D. Matula and R. sokal. Properties of gabriel graphs relevant to geographical variation research and the clustering of points in the same plane. *Geographical Analysis*, 12:205–222, 1984.

[201] R. Mayne. Biology of the Physarum polycephalum plasmodium: preliminaries for unconventional computing. In A. Adamatzky, editor, *Advances in Physarum Machines*, volume 21, chapter 1, pages 1–22. Springer, 2016.

[202] R. Mayne and A. Adamatkzy. Cellular automata modelling of slime mould actin network signalling. *Natural Computing*, pages 1–8, 2016.

[203] R. Mayne and A. Adamatzky. The Physarum polycephalum actin network: formalisation, topology and morphological correlates with computational ability. In *8th International Conference on Bio-inspired Information and Communications Technologies (formerly BIONETICS)*, pages 87–94, 2014.

[204] R. Mayne and A. Adamatzky. Towards hybrid nanostructure-artificial slime mould devices. *Nano LIFE*, 5(1), 2014.

[205] R. Mayne and A. Adamatzky. On the Computing Potential of Intracellular Vesicles. *Plos One*, 10(10):e0139617, 2015.

[206] R. Mayne and A. Adamatzky. Slime mould foraging behaviour as optically coupled logical operations. *International Journal of General Systems*, 44(3):305–313, 2015.

[207] R. Mayne, A. Adamatzky, and J. Jones. On the role of the plasmodial cytoskeleton in facilitating intelligent behaviour in slime mould Physarum polycephalum. *Communicative and Integrative Biology*, 8(4):e1059007, 2015.

[208] R. Mayne, J. Jones, E. Gale, and A. Adamatzky. On coupled oscillator dynamics and incident behaviour patterns in slime mould Physarum polycephalum: emergence of wave packets, global streaming clock frequencies and anticipation of periodic stimuli. *International Journal of Parallel, Emergent and Distributed Systems*, pages 1–24, 2016.

[209] R. Mayne, M.-A. Tsompanas, G. C. Sirakoulis, and A. Adamatzky. Towards a slime Mould-FPGA interface. *Biomedical Engineering Letters*, 5(1):51–57, 2015.

[210] C. McCullough and J. Dee. Defined and semi-defined media for the growth of amoebae of *Physarum polycephalum*. *Journal of General Microbiology*, 95(1):151–158, 1976.

[211] Medline Trends. Medline trends for search term 'physarum'. Available from `mltrends.ogic.ca`, 2016. [Accessed 22 Jan 2016].

[212] I. Meinertzhagen, S. Takemura, Z. Lu, S. Huang, S. Gao, C. Ting, and C. Lee. From form to function: the ways to know a neuron. *Journal of Neurogenetics*, 23:68–77, 2009.

[213] R. Meyer and W. Stockem. Studies on microplasmodia of physarum polycephalum v: electrical activity of different types of microplasmodia and macroplasmodia. 3(4):321–330, 1979.

[214] M. Miller and B. Bassler. Quorum sensing in bacteria. *Annual Review of Microbiology*, 55:165–199, 2001.

[215] H. Mo, L. Liu, and M. Geng. A new magnetotactic bacteria optimization algorithm based on moment migration. In Y. Tan, Y. Shi, and C. Coello, editors, *Advances in Swarm Intelligence*, volume 8794 of *Lecture Notes in Computer Science*, pages 103–114. Springer International Publishing, 2014.

[216] G. Moore. Cramming more components onto integrated circuits. *Electronics*, 38(8):114–117, 1965.

[217] Multi Channel Systems. Multi electrode array mea2100 system product information. Available from `http://www.multichannelsystems.com/products/mea2100-systems`. [Accessed 30 Aug 2016].

[218] T. Nakagaki. Smart behavior of true slime mold in a labyrinth. *Research in Microbiology*, 152(9):767–770, 2001.

[219] T. Nakagaki, H. Yamada, and A. Tóth. Maze-solving by an amoeboid organism. *Nature*, 407:470, 2000.

[220] T. Nakagaki, H. Yamada, and a. Tóth. Path finding by tube morphogenesis in an amoeboid organism. *Biophysical chemistry*, 92(1-2):47–52, aug 2001.

[221] S. Nakata, K. Kashima, H. Kitahata, and Y. Mori. Phase wave between two oscillators in the photosensitive belousovzhabotinsky reaction depending on the difference in the illumination time. *Journal of Physical Chemistry A*, 144(34):9124–9129, 2010.

[222] T. O'Connor and H. Wong. Emergent properties. In E. N. Zalta, editor, *The Stanford Encyclopedia of Philosophy*. 2015. [Accessed 16 Jan 2016.

[223] E. of Life. *Physarales* species distribution, 2015. Available from http://eol.org/pages/5756/maps [Accessed 08 July 2015].

[224] T. Oguchi and H. Nijmeijer. Anticipating synchronization of nonlinear systems with uncertainties. In *Proceedings of the 6th IFAC Workschop on Time-Delay Systems*, pages 290–295, 2006.

[225] K. Olenhusen and K. Wohlfarth-Bottermann. Evidence for actin transformation during the contraction-relaxation cycle of cytoplasmic actomyosin: cycle blockage by phalloidin injection. *Cell and Tissue Research*, 196:455–470, 1979.

[226] L. Olive. The mycetozoa: a revised classification. *The Botanical Review*, 36:59–89, 1970.

[227] L. Olsen and H. Degn. Chaos in biological systems. *Quarterly reviews of biophysics*, 18(2):165–225, 1985.

[228] K. Orphanides. Ibm's carbon nanotube breakthrough takes processors beyond silicon. WIRED magazine. Available from `http://www.wired.co.uk/news/archive/2015-10/02/ibm-carbon-nanotube-breakthrough-processors-silicon`, 2015. [Accessed 12 Jan 2016].

[229] A. Osbourn, P. O'Maille, S. Rosser, and K. Lindsey. Synthetic biology. *New Phytologist*, 196:671–677, 2012.

[230] P. Peltola, A. Ivask, M. Astrom, and M. Virta. Lead and copper in contaminated urban soils: extraction and with chemical reagents and bioluminescent bacteria and yeast. *Science of the Total Environment*, 350(1–3):194–203, 2005.

[231] R. Penrose. *The Emperor's New Mind*. Oxford University Press, 1990.

[232] Y. V. Pershin, S. La Fontaine, and M. Di Ventra. Memristive model of amoeba's learning. *Physical Review E*, 20(021926), 2009.

[233] C. Persoon. 'neuer versuch einer systematischen eintheilung der schwamme' [article in german]. *Neues Magazin fur die Botanik*, 1:63—128, 1794.

[234] R. Pfeifer and G. Gómez. Morphological computation — connecting the brain, body and environment. In B. Sendhoff, E. Körner, O. Sporns, H. Ritter, and K. Doya, editors, *Lecture Notes in Computer Science. Creating Brain-Like Intelligence*, pages 68–83. Springer, Berlin, 2009.

[235] R. Pfeifer, M. Lungarella, and F. Iida. Self-organization, embodiment, and biologically inspired robotics. *Science (New York, N.Y.)*, 318(5853):1088–93, nov 2007.

[236] Phychip Project. Project description. Available from `http://www.phychip.eu/`, 2016. [Accessed 24 Jan 2016].

[237] G. Pierron. *Proceedings of a NATO advanced research workshop on the molecular biology of* Physarum polycephalum, chapter 4: Temporal order of replication and gene expression. Plenum press, 1985.

[238] A. N. Pisarchik, R. Jaimes-Reátegui, and J. H. García-López. Synchronization of coupled bistable chaotic systems: experimental study. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, 366(1864):459–473, 2008.

[239] V. Privman. *Approaches to control of noise in chemical and biochemical information and signal processing*, chapter Twelve. John Wiley & Sons, 2013.

[240] D. Purves, G. Augustine, and D. Fitzpatrick, editors. *Increased conduction velocity as a result of myelination.* Second edition, 2001. Available from `http://www.ncbi.nlm.nih.gov/books/NBK10921/`. [Accessed 26 Jan 2016].

[241] T. Pyragiene and K. Pyragas. Anticipating spike synchronization in nonidentical chaotic neurons. *Nonlinear Dynamics*, 74(1-2):297–306, 2013.

[242] T. Pyragiene and K. Pyragas. Anticipating synchronization in a chain of chaotic oscillators with switching parameters. *Physics Letters A*, 379(47–48):3084–3088, 2015.

[243] M. Raichle and M. Mintun. Brain work and brain imaging. *Annual Review of Neuroscience*, 29:449–476, 2006.

[244] H. Rang, M. Dale, J. Ritter, and R. Flower. *Rang and Dale's Pharmacology*, chapter 4, page 57. Churchill Livingstone Elsevier, London, UK, 2007.

[245] H. Rang, M. Dale, J. Ritter, and R. Flower. *Rang and Dale's Pharmacology*, chapter 3, page 42. Churchill Livingstone Elsevier, London, UK, 2007.

[246] G. Rayfield. Kinetics of the light-driven proton movement in model membranes containing bacteriorhodopsin. *Biophysical Journal*, 38(1):79–84, 1982.

[247] C. Reid, T. Latty, A. Dussutour, and M. Beekman. Slime mold uses and externalized spatial "memory" to navigate in complex environments. *PNAS*, 109(43):17490–17494, 2012.

[248] R. Rhea. Electron microscopic observations on the slime mould *Physarum polycephalum* with specific reference to fibrillar structures. *Journal of Ultrastructure Research*, 15:349–379, 1966.

[249] J. Roerdink and A. Meijster. The watershed transform: definitions, algorithms and parallelization strategies. *Fundamenta Informaticae*, 41:187–228, 2001.

[250] R. Rosen. *Anticipatory systems: philosophical, mathematical and methodological foundations.* Springer, second edition, 1985.

[251] M. Rosenblum, A. Pikovsky, and J. Kurths. From phase to lag synchronization in coupled chaotic oscillators. *Physical Review Letters*, 78:4193–4196, 1996.

[252] M. Rosenblum, A. Pikovsky, and J. Kurths. Phase synchronization of chaotic oscillators. *Physical Review Letters*, 76:1804–1807, 1996.

[253] W. Sachsenmaier, J. Blessing, B. Brauser, and K. Hansen. Protoplasmic streaming in *Physarum polycephalum*: observation of spontaneous and induced changes of the oscillatory pattern by photometric and fluorometric studies. *Protoplasma*, 77:381–396, 1973.

[254] T. Saigusa, A. Tero, T. Nakagaki, and Y. Kuramoto. Amoebae Anticipate Periodic Events. *Physical Review Letters*, 100(1):018101, jan 2008.

[255] M. Santella, F. Amini, K. Andreasen, D. Aswad, H. Ausar, and *et al.* Template-guided ionic self-assembled molecular materials and thin films with nanoscopic order. *ChemNanoMat*, 1(4):256–258, 2015.

[256] P. Satir and S. Christensen. Overview of structure and function of mammalian cilia. *Annual review of physiology*, 69:277–400, 2007.

[257] H. Sauer. *Developmental biology of Physarum*. Cambridge University Press, Cambridge, UK, 1982.

[258] A. Schmidt and M. Hall. Signalling to the actin cytoskeleton. *Annual Reviews of Cell and Developmental Biology*, 14:305–338, 1998.

[259] M. Schuh. An actin-dependent mechanism for long range vesicle transport. *Nature Cell Biology*, 13(12):1431–1436, 2013.

[260] A. Schumann. Conventional and unconventional reversible logic gates on physarum polycephalum. *International Journal of General Systems*, 2015.

[261] A. Schumann and A. Adamatzky. Physarum spatial logic. *New Mathematics and Natural Computation*, 7(483), 2011.

[262] A. Schumann and A. Adamatzky. The double-slit experiment with physarum polycephalum and p-adic valued probabilities and fuzziness. *International Journal of General Systems*, 44(3):392–408, 2015.

[263] A. Schumann and K. Pancerz. Towards an object-oriented programming language for physarum polycephalum computing: A petri net model approach. *Fundamenta Informaticae*, 133(2–3):271–285, 2014.

[264] A. Schumann, K. Pancerz, A. Adamatzky, and M. Grube. Bio-inspired game theory: the case of *Physarum polycephalum*. In *8th International Conference on Bio-inspired Information and Communications Technologies (formerly BIONETICS)*, pages 9–16, 2014.

[265] B. Sengupta and M. B. Stemmler. Power consumption during neuronal computation. *Proceedings of the IEEE*, 102(5):738–750, 2014.

[266] K. Sharma and C. Munshi. A comprehensive and comparative study of maze-solving techniques by implementing graph theory. *IOSR Journal of Computer Engineering*, 17(1):24–29, 2015.

[267] F. Shih. *Image Processing and Mathematical Morphology: Fundamentals and Applications*. CRC Press, 2009.

[268] T. Shirakawa. Novel taxis of the physarum plasmodium and a taxis-based simulation of physarum swarm. In *Soft Computing and Intelligent Systems (SCIS) and 13th International Symposium on Advanced Intelligent Systems (ISIS)*, pages 296–300. IEEE Xplore, 2012.

[269] T. Shirakawa, A. Adamatzky, Y. Gunji, and Y. Miyake. On simultaneous construction of voronoi diagram and delaunay triangulation by *Physarum polycephalum*. 19:3109–3117, 2009.

[270] T. Shirakawa and Y. Gunji. Computation of voronoi diagram and collision-free path using the plasmodium of *Physarum polycephalum*. *International Journal of Unconventional Computing*, 6:79–88, 2009.

[271] P. Shor. Polynomial time algorithms for prime factorization and discrete logarithms on a quantum computer. *SIAM Journal on Computing*, 26(5):1484–1509, 1997.

[272] M. Shulaker, G. Hills, N. Patil, H. Wei, H. Chen, H. Wong, and S. Mitra. Carbon nanotube computer. *Nature*, 501:526–530, 2013.

[273] R. Silver, P. Balsam, M. Butler, and J. LeSauter. Food anticipation depends on oscillators and memories in both body and brain. *Physiology and Behaviour*, 104(4):562–571, 2011.

[274] P. Simons. The Role of Electricity in Plant Movements. *New Phytologist*, 87(1):11–37, 1981.

[275] V. Singh. Recent advancements in synthetic biology: current status and challenges. *Gene*, 535(1):1–11, 2014.

[276] G. Sirakoulis, I. Karafyllidis, C. Mizas, V. Mardiris, A. Thanaiilakis, and P. Tasalides. A cellular automaton model for the study of dna sequence evolution. *Computers in Biology and Medicine*, 33(5):439–453, 2003.

[277] T. Sjostrom and M. Weitzman. Competition and the evolution of efficiency. *Journal of Economic Behavior & Organization*, 30(1):25 – 43, 1996.

[278] D. Smith and R. Saldana. Model of the Ca2+ oscillator for shuttle streaming in Physarum polycephalum. *Biophysical Journal*, 61:368–380, 1992.

[279] H. Smith. Photoreceptors in signal transduction. *Plant Cell*, 12(1):1–4, 2000.

[280] H. Soga, S. Fujii, T. Yomo, Y. Kato, H. Watanabe, and T. Matsuura. In vitro membrane protein synthesis inside cell-sized vesicles reveals the dependence of membrane protein integration on vesicle volume. *ACS Synthetic Biology*, 3(6):372–379, 2014.

[281] D. Soll, D. Wessels, J. Murray, H. Vawter, E. Voss, and A. Bublitz. Intracellular vesicle movement, camp and myosin ii in dictyostelium. *Developmental Genetics*, 11(5–6):341–353, 1990.

[282] C. Starostzik and W. Marwan. A photoreceptor with characteristics of phytochrome triggers sporulation in the true slime mould *Physarum polycephalum*. *FEBS Letters*, 370:146–148, 1995.

[283] A. Steane. The ion trap quantum information processor. *Applied Physics B*, 64:623–642, 1997.

[284] S. Stephenson and H. Stempenson. *Myxomycetes: A handbook of slime moulds*. Timber Press, Portland, Oregon, USA, 1994.

[285] S. Stepney, S. Braunstein, J. Clark, A. Tyrrell, A. Adamatzky, R. Smith, T. Addis, C. Johnson, J. Timmis, P. Welch, R. Milner, and D. Partridge. Journeys in non-classical computation I: A grand challenge for computing research. *International Journal of Parallel, Emergent and Distributed Systems*, 20(1):5–19, 2005.

[286] D. Stoleru, Y. Peng, J. Agosto, and M. Rosbash. Coupled oscillators control morning and evening locomotor behaviour of *Drosophila*. *Nature*, 431:862–869, 2004.

[287] D. Strukov, G. Snider, D. Stewart, and S. Williams. The missing memristor found. *Nature*, 453:80–83, 2008.

[288] Y. Sudo and J. Spudich. Three strategically placed hydrogen-bonding residues convert a proton pump into a sensory receptor. *PNAS*, 103(44):16129–16134, 2006.

[289] T. Sugawara, M. Tachikawa, T. Tsukamoto, and T. Shimizu. Observation of synchronization in laser chaos. *Physical Review Letters*, 72:3502–3505, 1994.

[290] P. Suiti, J. Yazbek, and T. Lu. Synthetic circuits integrating logic and memory in living cells. *Nature Biotechnology*, 31:448–542, 2013.

[291] G. Tarabella, P. D'Angelo, A. Cifarelli, A. Dimonte, A. Romeo, T. Berzina, V. Erokhin, and S. Iannotta. A hybrid living/organic electrochemical transistor based on the physarum polycephalum cell endowed with both sensing and memristive properties. *Chemical Science*, 6:2859–2868, 2015.

[292] A. Tarakanov, V. Skormin, and S. Sokolova. *Immunocomputing: Principles and Applications*. Springer, 2003.

[293] J. Taunton, B. a. Rowning, M. L. Coughlin, M. Wu, R. T. Moon, T. J. Mitchison, and C. a. Larabell. Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. *Journal of Cell Biology*, 148(3):519–530, 2000.

[294] B. Taylor, A. Adamatzky, J. Greenman, and I. Ieropoulos. *Physarum polycephalum*: Towards a biological controller. *Biosystems*, 127:42–46, 2015.

[295] F. Tenore and R. Vogelstein. John hopkins apl technical digest. 30(3):230–239, 2011.

[296] The Slime Mould Collective. Available from `http://slimoco.ning.com/`, 2015. [Accessed 20 Jan 2016].

[297] T. Toffoli. *Symbol Super Colliders*, chapter 1, pages 1–26. Springer, London, UK, 2002.

[298] G. Tononi and C. Koch. Consciousness: here, there and everywhere? *Philosophical Transactions of the Royal Society B*, 370(1668), 2015.

[299] C. Torney, A. Berdahl, and I. Couzin. Signaling and the evolution of cooperative foraging in dynamic environments. *PLoS Computational Biology*, 7(9):e1002194, 2011.

[300] G. Toussaint. The relative neighbourhood graph of a finite planar set. *Pattern Recognition*, 12:261–268, 1980.

[301] M. Tsompanas, R. Mayne, G. Sirakoulis, and A. Adamatzky. A cellular automata bioinspired algorithm designing data trees in wireless sensor networks. *International Journal of Distributed Sensor Networks*, 2015.

[302] S. Tsuda, M. Aono, and Y. Gunji. Robust and emergent physarum logical-computing. *Biosystems*, 73(1):45–55, 2004.

[303] S. Tsuda, K. Zauner, and Y. Gunji. Robot control with biological cells. *Biosystems*, 87:215–223, 2007.

[304] A. Turing. Intelligent machinery, a heretical theory (reprint). *Philosophia Mathematica*, 4(3):256–260, 1996.

[305] S. Tusda, J. Jones, and A. Adamatzky. Towards physarum engines. *Applied Bionics and Biomechanics*, 9(3):221–240, 2012.

[306] K. Tusscher and A. Panfilov. Wave propagation in excitable media with randomly distributed obstacles. *Multiscale Modelling and Simulation*, 3(2):265–282, 2005.

[307] J. Tuszyński, S. Portet, J. Dixon, C. Luxford, and H. Cantiello. Ionic wave propagation along actin filaments. *Biophysical Journal*, 86:1890–1903, 2004.

[308] J. Tyson. *The Biology of* Physarum *and* Didymium, volume 1 of *Cell Biology: A series of monographs*, chapter 3: Periodic phenomena in *Physarum*. Academic Press, 1982.

[309] T. Ueda and K. Götz Von Olenhusen. Replacement of endoplasm with artificial media in plasmodial strands of *Physarum polycephalum*: Effects on contractility and morphology. *Experimental Cell Research*, 116(1):55–62, 1978.

[310] T. Ueda, T. Hirose, and Y. Kobatake. Membrane biophysics of chemoreception and taxis in the plasmodium of *Physarum polycephalum*. *Biophysical Journal*, 11:461–179, 1980.

[311] T. Ueda and Y. Kobatake. *The Biology of* Physarum *and* Didymium, volume 1 of *Cell Biology: A series of monographs*, chapter 4: Chemotaxis in plasmodia of *Physarum polycephalum*, pages 101–131. Academic Press, 1982.

[312] T. Ueda, K. Matsumoto, T. Akitaya, and Y. Kobatake. Spatial and temporal organization of intracellular adenine nucleotides and cyclic nucleotides in relation to rhythmic motility in *Physarum* plasmodium. *Experimental Cell Research*, 162:486–494, 1986.

[313] J. Van Der Meer and S. Belkin. Where microbiology meets microengineering: design and applications of reporter bacteria. *Nature Reviews Microbiology*, 8:511–522, 2010.

[314] P. Van der Sluijs, M. K. Bennett, C. Antony, K. Simons, and T. E. Kreis. Binding of exocytic vesicles from MDCK cells to microtubules in vitro. *Journal of cell science*, 95 (Pt 4)(1986):545–553, 1990.

[315] L. Vandersypen, M. Steffen, G. Breyta, C. Yannoni, M. Sherwood, and I. Chuang. Experimental realization of shor's quantum factoring algorithm using nuclear magnetic resonance. *Nature*, 414(6866):883–887, 2001.

[316] M. Vinzenz, M. Nemethova, F. Schur, J. Mueller, a. Narita, E. Urban, C. Winkler, C. Schmeiser, S. a. Koestler, K. Rottner, G. P. Resch, Y. Maeda, and J. V. Small. Actin branching in the initiation and maintenance of lamellipodia. *Journal of Cell Science*, 125:2775–2785, 2012.

[317] J. Von Neumann. *Theory of self-replicating automata*. University of Illinois Press, IL, USA, 1996.

[318] H. Voss. Anticipating chaotic synchronization. *Physical Review E*, 61:5115–5119, 2000.

[319] X. Walter, I. Horsfield, R. Mayne, I. Ieropoulos, and A. Adamatzky. On hybrid circuits exploiting thermistive properties of slime mould. *Nature Scientific Reports*, 6:23925, 2016.

[320] A. Waugh and A. Grant. *Anatomy and Phyiology in Health and Illness*. Churchill Livingstone Elsevier, tenth edition, 2007.

[321] W. Weber and M. Fussenegger. Emergin biomedical applications of synthetic biology. *Nature Reviews Genetics*, 13:21–35, 2012.

[322] J. Whiting, B. de Lacy Costello, and A. Adamatzky. Sensory fusion in *Physarum polycephalum* and implementing multi-sensory functional computation. *Biosystems*, 119:45–52, 2014.

[323] J. Whiting, B. de Lacy Costello, and A. Adamatzky. Slime mould logic gates based on frequency changes of electrical potential oscillation. *Biosystems*, 124:21–25, 2014.

[324] J. Whiting, B. de Lacy Costello, and A. Adamatzky. Towards slime mould chemical sensor: mapping chemical inputs onto electrical potential dynamics of *Physarum polycephalum*. *Sensors and Actuators B*, 191:844–853, 2014.

[325] J. Whiting, R. Mayne, N. Moody, B. D. L. Costello, and A. Adamatzky. Practical circuits with physarum wires. *Biomedical Engineering Letters*, Accepted for publication 2 Jan 2016, 2016.

[326] J. Willey, L. Sherwood, and C. Woolverton. chapter 1, page 2. McGraw Hill, NY, USA, 2008.

[327] J. Willey, L. Sherwood, and C. Woolverton. chapter 25, page 605. McGraw Hill, NY, USA, 2008.

[328] J. Willey, L. Sherwood, and C. Woolverton. chapter 3, page 605. McGraw Hill, NY, USA, 2008.

[329] A. Winfree. *The geometry of biological time*. Springer-Verlag, Berlin, Germany, 1980.

[330] K. Wohlfarth-Bottermann. Plasmalemma invaginations as characteristic constituents of plasmodia of Physarum polycephalum. *Journal of Cell Science*, 16(1):23–37, 1974.

[331] K. Wohlfarth-Bottermann. Tensiometric demonstration of endogenous oscillating contractions in plasmodia of *Physarum polycephalum*. *Zeitschrift für Pflanzenphysiologie*, 76:14–27, 1975.

[332] K. E. Wohlfarth-Bottermann. Oscillating contractions in protoplasmic strands of Physarum: simultaneous tensiometry of longitudinal and radial rhythms, periodicity analysis and temperature dependence. *The Journal of experimental biology*, 67:49–59, apr 1977.

[333] K. E. Wohlfarth-Bottermann. Oscillatory contraction activity in Physarum. *The Journal of experimental biology*, 81:15–32, 1979.

[334] R. Wolf, J. Niemuth, and H. Sauer. Thermotaxis and protoplasmic oscillations inPhysarum plasmodia analysed in a novel device generating stable linear temperature gradients. *Protoplasma*, 197:121–131, 1997.

[335] S. Wolfram. Universality and complexity in cellular automata. *Physica D*, 10:1–35, 1985.

[336] S. Wolfram. chapter 6, page 282. Wolfram Media, Illinois, USA, 2002.

[337] N. Woolf, A. Priel, and J. Tuszyski. The cytoskeleton as a nanoscale information processor: electrical properties and an actin-microtubule network model. In N. Woolf, A. Priel, and J. Tuszynski, editors, *Nanoneuroscience. Structural and Functional Roles of the Neuronal Cytoskeleton in Health and Disease*, pages 85–127. Springer, Berlin, 2010.

[338] W. Woolf. *Modern VLSI Design*. Prentice Hall PTR, NJ, USA, 2002.

[339] W. Wormington and R. Weaver. Photoreceptor pigment that induces differentiation in the slime mold *Physarum polycephalum*. *Proceedings of the National Academy of Sciences of the USA*, 73(11):3896–3899, 1976.

[340] A. Wuensche. *Finding gliders in cellular automata*, chapter 13, pages 381–410. Springer, 2002.

[341] J. Yang, Y. Jung, J. Chang, R. Mickiewicz, A. Alexander-Katz, C. Ross, and K. Berggren. Complex self-assembled patterns using sparse commensurate templates with locally varying motifs. *Nature Nanotechnology*, 5:256–260, 2010.

[342] S. Yantis. *Sensation and Perception*. Worth Publishers, New York, USA, 2013.

[343] Y. Yoshimoto and N. Kamiya. Studies on contraction rhythm of the plasmodial strand. *Protoplasma*, 95(89–97), 1978.

[344] Y. Yoshimoto, F. Matsumura, and N. Kamiya. Simultaneous oscillations of ca2$^+$ efflux and tension generation in the permelized plasmodial strand of *Physarum*. *Cell Motility*, 1(4):433–434, 1981.

[345] Y. Yoshimoto, T. Sakai, and N. Kamiya. Atp oscillation in *Physarum* plasmodium. *Protoplasma*, 109:159–168, 1981.

[346] S. Yoshiyama, M. Ishigami, A. Nakamura, and K. Kohama. Calcium wave for cytoplasmic streaming of Physarum polycephalum. *Cell biology international*, 34(1):35–40, Jan. 2010.

[347] A. Zhabotinsky, M. Eager, and R. Epstein. Refraction and reflection of chemical waves. *Physical Review Letters*, 71:1526–1529, 1993.