Physarum, Quo Vadis?

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Abstract In the recent years, computer scientists have been inspired by biological systems for computational approaches, in particularly with respect to complex optimization and decision problems. Nature provides a wealth of evolved solutions to such challenges. As evolved by natural selection, biological processes are robust and able to successfully handle failures as well as attacks to survive and propagate. Biological systems are mostly distributed systems that coordinate to make decisions without central control. An example *par excellence* for such a biological system is given by slime molds. In this context, *Physarum polycephalum* emerged as a model organism which has attracted substantial interest in the recent years. In this chapter, I present new approaches to cultivate this organism, with the goal to establish a multipurpose experimental platform for biological information processing.

1   Introduction

Physarum polycephalum is an acellular slime mold, which initially starts to grow as single-celled amoebae. After compatible amoebae fuse, the resulting organism starts to grow without cell division but with continued multiplication of nuclei every 8–10 h (coenocytic stage). The progressing growth leads to a huge polymorphic cell known as a macroplasmodium (Fig. 1). An external matrix of glycoprotein is giving this organism its characteristic slimy appearance. Naturally, slime mold plasmodia typically appear in moist environments such as damp forests or in alpine heaths after snow melt. In their natural environments they feed on other microbes or mushrooms present in leaf litter and other decaying plant matter, in bark, and other substrates.

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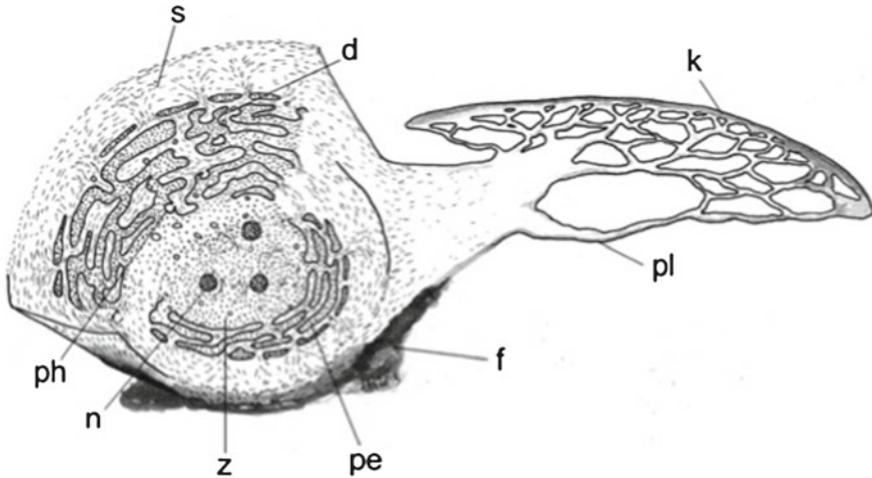
Fig. 1 Schematic drawing of slime mold macroplasmodium with facing side sectioned (and with *upper left* section enlarged); *k* moving front, *pl* trailing plasmodial strand, *f* deposited material, *s* slime; *d* vacuole with deposit; *ph* phagocytosis vesicle; *n* nucleus; *z* central plasma; *pe* peripheric membrane stacks. Peristaltic contractions occur in the peripheric plasma, while the central plasma is subject to shuttle streaming (Drawing by the author)

The typical macroplasmodium of *P. polycephalum* is organized as a tubular network, where the coherent internal cytoplasma can freely move and is surrounded by connected layers of peripheric cytoplasma. The network is perpetually reshaped to adapt to the environmental conditions and to move the entire body. The extraordinary morphological plasticity is achieved by periodic peristalsis of the ectoplasma due to cross-sectional, acto-myosin based contractions. These contractions cause the streaming of cytoplasma through the network. The streaming of cytoplasma changes its direction every 50 ± 5 s, and can reach speeds of up to 1 mm/s in the thickest tubes but can be significantly slower is narrower tubes and in the growing edges where it naturally encounters environmental triggers. Signaling molecules and nutrients are thus rapidly transmitted by the peristaltic waves as analogues of information through the tubular network using this mechanism of shuttle streaming. The length of the peristaltic wave is actively matched to the organismś size, so that points of zero flow velocity are effectively eliminated [2], and the net transport of cytoplasm through the entire network also moves the organism as a whole. As the plasmodium moves, it can grow depending on the nutrient availability.

When nutrient take-up diminishes in relation to other parts of the plasmodium, tubes are thinning and retracted residual content is transported towards active parts. This process leaves extracellular deposits behind, which remain as tracks of the former network. The extracellular slime represents high molecular weight, polyanionic glycoproteins [10], which are composed of largely of sulfated galactose polymers [12], and a D-galactan, partially substituted by sulfate and phosphate groups [7]. In Physarum, the moving plasmodium avoids to grow into areas of conspecifics

containing tracks of such deposits. By some authors, the repelling deposits have been interpreted a kind of extracellular spatial memory of Physarum [14], by which the organisms seems to recall the already foraged regions of the substrate.

2 Phalanx and Guerrilla Strategies—Two Basic Growth Styles

If Physarum is properly supplied with nutrition it extends with a broad, coherent, and dense front zone, which is linked to a transition zone and trailed by an extended network of tubular veins. However, the moving front tends to develop variably sized outgrowths (fingers), at an extent that is negatively correlated with the velocity of the entire growing front [3]. With depleting nutrient availability, finger formation becomes more pronounced and the formation of a coherent growing front is given up and replaced by a branched extension of the tubular network with multiple tips. In this condition, the plasmodium of Physarum resembles somehow the branching network of foraging fungal hyphae. Hence, two substantially different strategies of network development can be distinguished, which are here termed 'Phalanx' and 'Guerrilla' strategy. The Phalanx strategy is followed when Physarum growth is propelled by sufficient amount of nutrients. The development of a tubular network is then achieved after the growing front moved over the substrate by reduction of the biomass to develop a parsimonious transport system between the growing edge and the rest of the plasmodium. This reductional process is also the basis of Physarum experiments to find shortest path solutions, for example the maze-solving experiment [13] or more complex problems, such as approximation and evaluation of transport networks [17]. Contrarily, in the Guerrilla stage, Physarum tends to develop thin branches (pseudopodia) instead of a growing front for exploration. This behavior allows efficiently exploration of its environment for more distant nutrient sources. Chemical attractants sensed by the individually branched tips direct growth effectively to potential nutrient sources and which represent adaptive search strategies. The network is here formed without the presence of a closed growth front. The Phalanx and Guerilla growth strategies represent edge types, and intermediary styles are often encountered both in nature as well as in experimental set-ups [15]. Somehow similar, but not entirely comparable, are foraging strategies of predatory army ants, where the term 'swarm raid' describes a fan-shaped closed front, similar to the phalanx strategy, with a trailing path system, and the 'column raid' with several columns of scouting ants [9]. Alternatively, network development can also be achieved by fusion, e.g., the formation of an extended tubular network by fusion of so-called microplasmodia on agar [8]. In fact, microplasmodia are small spheric versions of plasmodia, which have been produced by shaking and disrupting liquid cultures of plasmodia in sem-idefined media at a speed of 300 rpms. Interestingly the plated microplasmodia reveal an overlay of oscillations with frequencies of around 100 s but also several minutes [4].
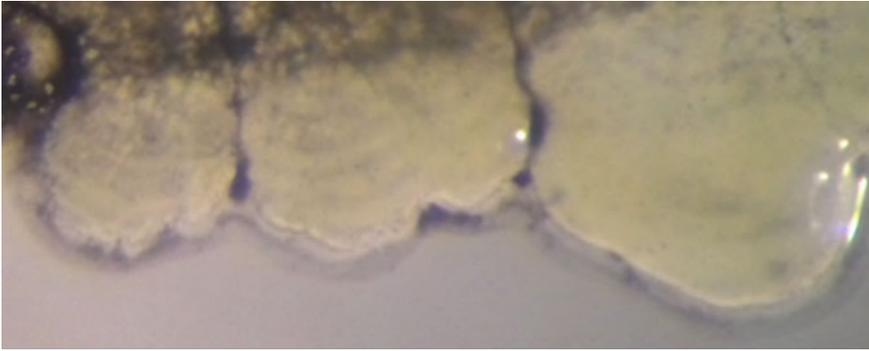
Fig. 2 Visualisation of periodic growth of Physarum using carbon particles. Particles ingested elsewhere by Physarum are deposited periodically at the growing edges. Note the growing edge is formed by several fingers, which are hardly distinguishable without the loading procedure. The distance between the bands is about $30\,\mu$m

Irrespective of the plasmodial growth strategies, the vein system is typically extended at the growing edge every 100 ± 10 s due to the forces of the shuttle streaming. This stepwise growth can readily be visualized using stroboscopic analysis of images or by loading the plasmodium with contrasting particles. An example of this technique is presented in Fig. 2, which was produced in a carbon particle uptake experiment. Carbon particles where ingested at on part of the plasmodium and then effectively deposited during phases of backward shuttle streaming, and thereby giving rise to a distinctive banding pattern at the growing edge.

3 Network Optimization of Physarum

As the plasmodium grows it adjusts it networking body to the accessed resources. Latty and Beekman [11] suggest a positive effect of food quality on fractal dimension of the searching network and suggest that the amount of localized search performed by plasmodia increased with food quality. Physarum also weighs its tubular structure when nutrients are split into carbon-rich and protein rich fractions [6], suggesting that the organism can make complex nutritional decisions to adjust its network in the precise proportions necessary to compose an optimal diet. The integration and accumulation of information and positive feedback on the tube diameter adjustments recall learning from the environmental cues, which appears to be a natural implementation of a memristor. The network structure is highly resilient to disturbances and if a tube is defunctionalized, flow can be directed through alternative routes, which then adjust rapidly by changing the tube width (changes of tube diameter result in cubic increase of flow). This behavior is technically strategy of fault tolerance, evolved by nature as to avoid death of the entire organism by injuries. Network

Fig. 3 Possible fates of cut or damaged plasmodial tubes, depending on tube width and distance of dissected ends
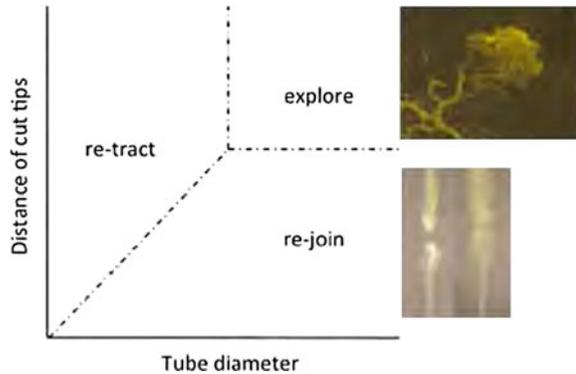
flow is stopped immediately when the network is touched or cut, a reaction to avoid complete leakage of cell content from the tubes. Slight touches of the plasmodium cause a rest in the local flow only and flow restores after some minutes, meanwhile causing and re-organisation of the flow in the surrounding portions. As changes in flow and electric current after mechanic stress can be measured, this property is the basis to propose Physarum as a touch sensor [1]. In contrast, the injury or entire cut of a plasmodial tube leads to rapid sealing of the resulting tips. Depending on the extent of the damage and the width of the tubes, they experience several fates: the separated tubes may (1) fuse again, (2) re-route to connect with other parts of the plasmodium, (3) explore the vicinity independently, or (4) one or both are retracted (Fig. 3).

All these studies of network optimization by the slime mold have been conducted with unconstrained conditions of water accessibility, usually on media (or on other water soaked supports, including paper) in Petri dishes or other closed containments. Therefore, the effect of water availability and air humidity on plasmodial architecture—as trivial as this might seem—has hardly been assessed. In closed Petri dishes, the air humidity ranges between 95–98 % (as measured by humidity sensors), which appears optimal for growth. As soon as the lid of a containment such as a Petri dish is lifted, air humidity immediately drops to values below 70 %, which exposes Physarum to an immediate desiccation shock, unless the lid is closed immediately again to restore tolerable humidity as soon as possible, in the range of several minutes. Taller containments may more effectively keep higher levels of humidity. Extended low air humidity affects initially the flow in narrow tubes of the plasmodial networks, which results in retraction of tinier tubes and a shift to broader average tube width. Thus, plasmodia are effective humidity sensors as they modify the network structure in response to fluctuations of air humidity. Perpetuated strong desiccation typically leads to formation of a sclerotium, as the vegetative resting stage of the plasmodium.

Water content on media for growth of planar plasmodia also has significant consequences on network structure. The Phalanx type morphology is preferentially developed on solid agar, especially if the is supplied with homogeneously distributed nutrients, e.g. oat flake extract [15]. In contrast, own experiments conducted with

water agar plugs surrounded by water in a Petri dish usually showed that plasmodia extending to water develop extended tubes with branches, but with increased nutrient conditions in water, tubes become thicker and more richly branched. Some growth experiments ("landscape foraging experiments") on solid agar surfaces have been conducted with varied distribution of nutrient sources [11]. As the plasmodium shifts from extensive to intensive search in presence of localized nutrients, correlated landscapes with grouped food increased better in weight than uncorrelated food landscapes.

These and similar other experiments also show that Physarum grows with pseudopodia towards the food resources, either nutrient-rich agar plugs (food disks) or spread oat flakes. With decreasing distance to food sources Physarum shifts from a more or less serendipitous search to a directed growth. A series of experiments, where oat flakes are removed before Physarum is able to establish contact reveals that this phenomenon is most likely due to a diffusible fraction of nutrients or attractants. However the effects of diffusible regulators of growth have so far little been studied in Physarum although this might be of profound influence on how network structures are developed, and on how Physarum might behave on growth supports on which diffusion is limited.

4 The Concept of a Chip

For applications it interesting to achieve a directed growth of Physarum between contact points as a basis for localized measurements. A series of papers already investigated Physarum tubes connecting two points for electrical measurements (e.g., [1]). For more complex electrical or optical measurements it is therefore useful to devise a growth platform for more complex directed growth of network tubes. Several plastic materials were tested for growth with Physarum, including plexiglass, polystyrol, polyaniline, and polyacetal. Clear differences were observed with respect to growth and network development of Physarum. Plane plexiglas was less amenable for growth than polyacetal, but scoring of the plexiglass surface made an interesting difference. Physarum has the tendency to track the scores on the acrylic surface, which suggests a kind of thigmotropic behaviour. Growth could thereby be controlled to good extent. Plexiglass squares of $6 \times 6 \times 0.5$ cm size, which fit in standard Petri dishes, were then cut out of larger plates and a rectangular grid-like pattern was then carved on the upper surface with 0.5 mm deep channels (Fig. 4). The spacing of the grid carving was 2.5 mm. At the crossing points of the grids round pits of c. 1 mm diameter and depth were then milled using a drill. The finished plate was used for inoculation with Physarum. To meet the requirement of high humidity for growth, the plates were placed in a Petri dish which, was then supplied with c. 50 ml of pure water at the sides of the inserted plates to reach air humidity of more than 95 %.

Fig. 4 Carved plexi glass plate for Physarum experiments. Overview (**a**), and rectangular development of Physarum plasmodium (**b**). Note the expanding part in the *upper* part of the *right* panel with fan-like protrusions, and a branching in the 4th pit
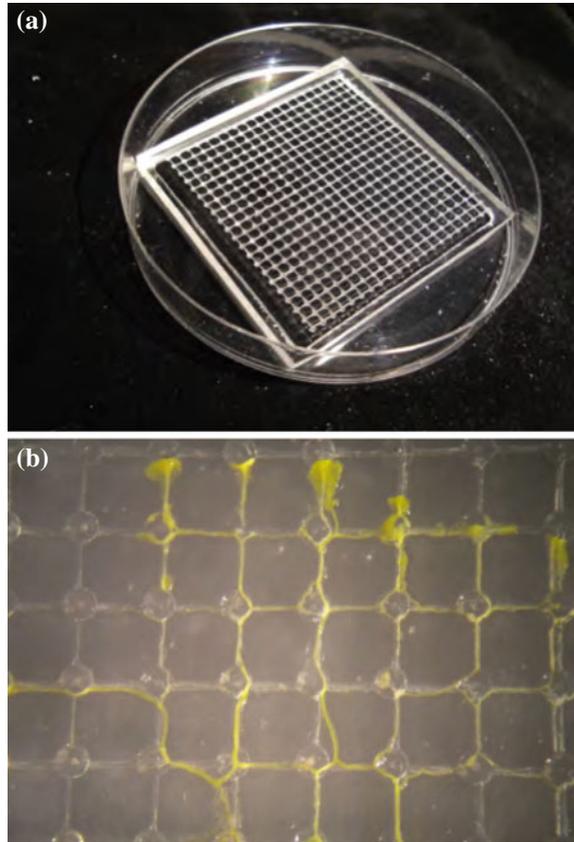
Plates were then inoculated with Physarum, supplied on $3 \times 3 \times 3$ mm agar plugs in the center of the plate, to develop a network of rectangular junctions. At the junctions of the carvings, which are widened as pits, the slime mold eventually forms turns, and/or branching at 90° angles to the left or right. The growth direction was erratic in the absence of additional nutrient supply. Network tubes are enforced when connections were established between pits supplied with nutrients (for experimentation 1 µl oat slurry was supplied to pits using a micropipette). If all four junctions are connected, a ring-link structure emerges (recalling a bidirectional roundabout; Fig. 5). Cytoplasmic content flows through the connected tubes in several configurations, which can be described as a vector, using the direction of flow in junctions a, b, c, d as values (e.g.: 0, 1, 0, 1). Some configurations are hardly possibly, such as 1, 1, 1, 1 or 0, 0, 0, 0, whereas other configurations prove to follow a complex timings, as flows are not simply reverted after the average half phase time of 50 ± 5 s. This instability is partly explained by the fact that Physarum is still moving at its fronts (here by independent protrusions in the grid layout), but also by regulatory triggers such as local illumination.
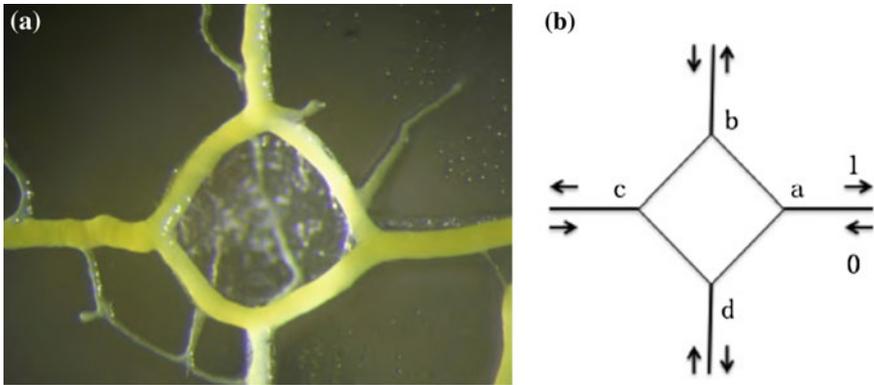
Fig. 5 Four-way switch of Physarum tubes on plexiglass grid (**a**) and schematic representation of I/O possibilities (**b**)

The perpetual growth of the Physarum, especially with the presence of nutrients, which propels the organism with energy, leads the plasmodium to naturally escape the plates, by growing toward the periphery or by sensing the surrounding humidity. This proves to be a challenge for providing a stable condition for computation experiments using plasmodial flows. Escape from the plates usually occurs within two days, and attempts are underway to prevent escape for extended periods.

Alternatively, smaller tiles for Physarum network growth were chosen. For this purpose it was found that microscopic cover lids were optimal as Physarum readily grows networks on glass if air humidty was appropriately high. This requirement was met by placing cover lids in Petri dishes, and then inoculating the lids with Physarum before closing the Petri dish. This resulted in tiny networks of Physarum, which readily extended over the glass slide. As these networks are highly sensitive to drops in air humidity, opening of the Petri dish lids was largely avoided during experimentation. It was repeatedly found that air humidity drops by opening may result in fragmentation of the coherent networks. To avoid such stress even at start of experiments, plasmodium inoculation was conducted by dropping small plasmodial portion in the center of the glass slides. This was achieved by placing a cuneiform piece of plasmodium-colonized agar on the lid of the Petri dish. Owing to the negative geotropism the plasmodium formed a small pseudopodium which extends downwards and which could precisely oriented to contact the center of the glass slide. A small turn of the Petri dish lid then disrupts the connection with the residual plasmodium and a small plasmodial portion was dropped to the center of the glass slide without any effect to air humidity. The dropped plasmodium started growth on the glass plate within minutes to explore the vicinity (Fig. 6).

Similar to the larger plexi glass supports, the plasmodia tended to rapidly escape the glass slides. However, escape of Physarum into the surrounding medium was prevented here prevented by (a) placing the glass slide on a slightly larger sheet of transparency foil (cellulose acetate) and (b) by constant illumination of the periphery
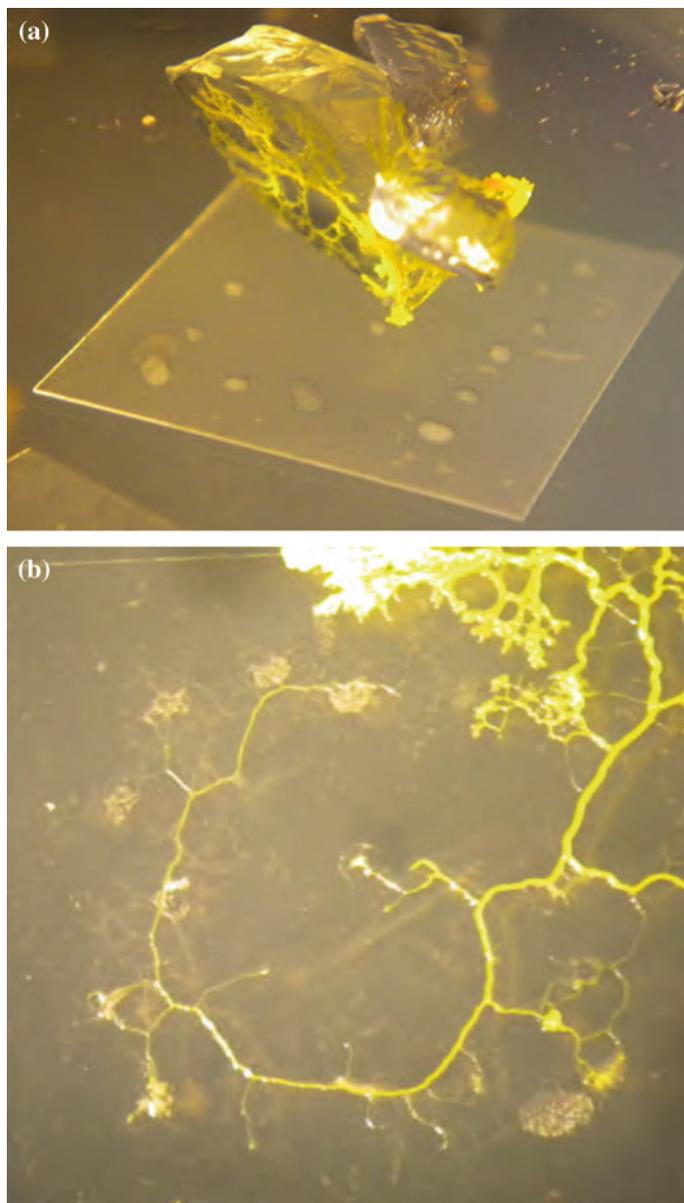
Fig. 6 Inoculation of plasmodial portion on glass slide and plasmodial exploration. *Left panel* agar plug mounted on the cover of a Petri dish, positioned with its tip to the center of the glass slide. A pseudopodium start downward growth to finally contact the slide. *Right panel* Network developed from a centrally placed pseudopodium. Residuals of contacted oat slurry dots are recognized as whitish spots. After 12 h the plasmodium has contacted virtually all dots (except the one on the *lower right*), and extended growth anticlock wise to reach the periphery of the glass slide
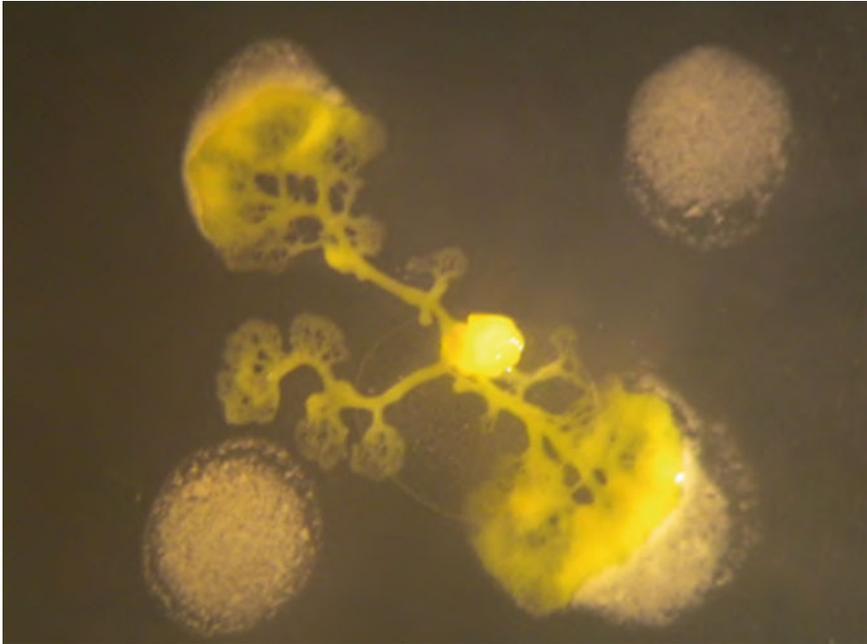
Fig. 7 Physarum pseudopodium dropped on a glass slide explores the vicinity. White plugs represent oat slurry. The plug in the *upper right corner* was constantly illuminated, whereas the plug to the *lower left* was illuminated periodically with 30 s darkness alternating with 30 s illumination

of the glass slide, which was achieved by gluing a piece of appropriately sized black and non-translucent paper on the lower side of the Petri dish containing the glass slide, to avoid unnecessary illumination of the glass slides. Next, behavior of glass slide-exploring plasmodia was explored in the presence of localized light. Light, particularly white and blue light, generally seems to affect the growth of plasmodia [13]. White light diodes were conducted to glass fiber to precisely illuminate small portions of the glass slide, either with constant light or with periodic light, in the present experiment alternating every 30 s. Physarum readily contact non-illuminated spots of oat slurry (1 μl of slurry placed dot-wise in equidistance of 3 mm to the inoculum; the slurry was prepared by crudely mixing two flakes of oat with 200 μl of pure water), whereas it avoids to contact dots either continuously illuminated or periodically illuminated (Fig. 7).

This approach proved efficient triggering of plasmodial growth in a miniaturized set-up, which could be used for more complex optimization tasks, which varying chemical, optical or electric triggers. It should be emphasized that the here present set-up, in contrast to more traditional agar-based experiments exclude any effects due to diffusible compounds through the medium. The interfacing with conventional information processing technology is at the present stage best achieved by optical measurements and image analysis.

Experiments in the context of decision making by slime molds, as presented here, are mostly approaches in 'one go' (until the plasmodium explored the whole experimental area). Physarum, exposed to nutritional cues and triggered by light, aims to search for an optimal solution by nonrecurring plasmodium arrangement. Once this is achieved by growth the experiment is finished and solutions can be read out. The same chip, either in form of a plexiglass plate or a miniature glass slide, is thus limited to a single experiment that is then usually discarded. The only alternatives of somehow extended use of Physarum for computational devices is envisioned by [16], which employ Physarum plasmodia as a steady material in an electronic device. However, it remains to be elaborated, whether grown networks of Physarum might be used in other contexts. Physarum networks are grown solutions over time, which do not only consist of the growing part, but also of the traces left behind. These traces are the stored historic attempts to find a solution to a path problem, and as such, these follow a general program of search strategy scripted in the genome of the searching organism. As the slime tracks of Physarum are acidic, they can be stained with the appropriate histochemical dyes. Acidic moieties are usually stained by thiazine dyes, such as Toluidine Blue O (in 1 % aquous solution). This proved to be a highly efficient dye to contrast the plasmodial tracks, which correlate with the width of the tubes. Toluidine Blue staining of plasmodial tracks resulted in metachromatic colour shifts, from orthochromatic blue of the dye, towards red colours (as the closely spaced acidic groups of molecules in the tracks cause dimeric to polymeric aggregates of the dye and their interaction via pi-orbitals). This way highly contrasted images of the slime mold network could be obtained for subsequent image analysis. For a first example, the NEFI program provides an ideal pipeline to process images of slime mold networks [5]. Using NEFI, the images were preprocessed and analysed to provide an overall description of network properties (Fig. 8).

The structure of networks in Fig. 7 show slight differences, depicted as an example by the histogram of tube (plus deposit) width, which differ by the slope towards wider tubes. Further experiments need to be conducted to further describe general differences between networks. These difference may results from different nutritional stages of the inoculum and from the present growth conditions. It should be noted also that the different strains of Physarum have slightly different exploitation strategies [18]. Thus, there might be slight variations in network properties among slide-grown Physarum experiments that are stored by the stained network. It is suggested that these fixed networks be on-chip memories of their search feature. What could be a use of these chips? It might eventually be possible to supply environment-exploring robots with slime mold-based search parameters. Owing to variations found in slime mold networks it will then be possible to provide robots with some kind of individuality, which could be useful for optimization of robots socially acting in swarms.
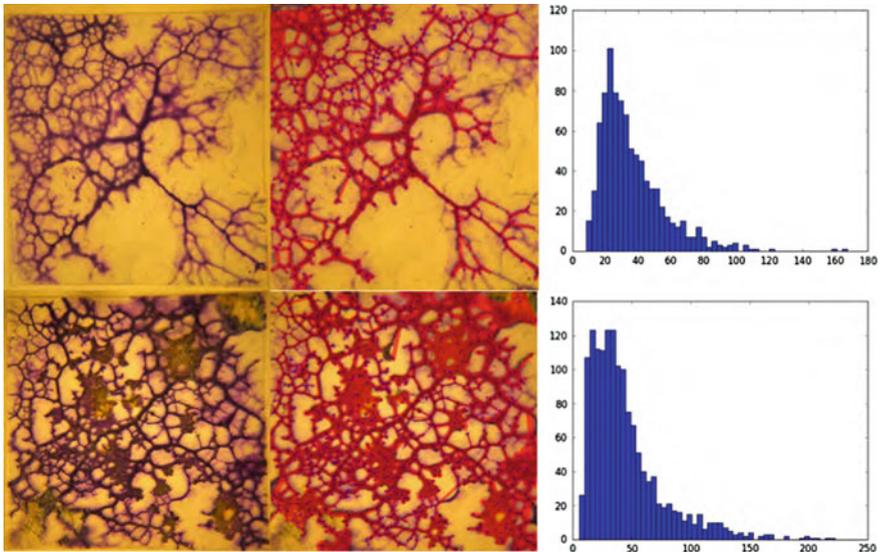
Fig. 8 Example of image processing using NEFI. Two exemplary networks of Physarum were grown, *Upper rows* growth without nutrients; *Lower Row* glass slide supplied with several nutrient spots. *Left panel* original image of Physarum network stained with 1% Toluidine Blue. *Middle panel* Preprocessed image with segmentation. *Right panel* Exemplary histograms of tube width. Explanation in text

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