A close look at amoeboid locomotion: An integrated picture of a migrating, starvation-induced foraging unit of *Physarum polycephalum*

Christina Oettmeier
Institut für Biophysik
University of Bremen
Germany
coettmeier@biophysik.uni-bremen.de

Hans-Günther Döbereiner
Institut für Biophysik
University of Bremen
Germany
hgd@biophysik.uni-bremen.de

ABSTRACT

Physarum polycephalum, cultivated on a glucose-deficient agar surface, forms disconnected foraging units (satellites). Our aim is to shed light onto the amoeboid locomotion of the slime mould, using satellites as reproducible and well-defined models and employing a wide range of techniques. This work was presented at PhysNet 2015.

Categories and Subject Descriptors

J.3 [**Computer Applications**]: Life and Medical Sciences—*Biology and Genetics*

Keywords

Physarum polycephalum, shuttle streaming, amoeboid locomotion

1. INTRODUCTION

The slime mould *Physarum polycephalum* forms an extended vein network which serves to distribute fluid cytoplasm (termed endoplasm) containing nutrients, chemicals and cellular components throughout the cell body (plasmodium). The flow of endoplasm through the veins of the network, whose walls consist of solid ectoplasm, is achieved through an oscillatory mechanism called shuttle streaming. The shuttle streaming is not organized randomly, but moves like a peristaltic wave throughout the network [1].

To create reproducible, small units of the slime mould, we transfer a plasmodium into a liquid shaking culture. Shear then creates disconnected microplasmodia [2], which begin to migrate and fuse with each other when placed in patches on a 2-dimensional agar substrate. When enough nutrients are present, these microplasmodia fuse in a percolation transition directly into an extended network [3]. Under certain circumstances, namely when glucose is lacking from the agar and when the microplasmodia culture has a certain age, so-called satellites are formed. We conjecture that those disjunct, autonomous units represent a foraging strategy. In contrast to the larger network, they migrate at a constant speed and maintain a straight trajectory away from the original patch. We use satellites as a model system to study the amoeboid locomotion of *P. polycephalum*. Satellites have a persistent shape and size, which makes the analysis of the periphery relatively simple. In addition, they are not part of an extended network, but self-contained. Due to their thinness (a satellite is only 100 to 200 μm in height), internal cytoplasm dynamics are well visible under a light microscope.

Amoeboid locomotion has been described for many cell types, but the exact mechanism remains unknown [4]. In *P. polycephalum*, contributing factors are the acto-myosin cytoskeleton, shuttle streaming, adhesion to the substrate and chemical gradients, e.g., of calcium ions. We investigate these factors with various light- and electron microscopic techniques and propose an integrated picture of amoeboid locomotion in *P. polycephalum*.

2. CONTOUR DYNAMICS AND INTERNAL FLOW

Steadily moving satellites growing on an agar surface are recorded under a microscope. Frame rates are chosen to be very high, in the range of several ten images per second, to account for the fast flow velocity (approximately 20 μm/s) inside the satellites. The resulting time series with a high temporal resolution is then used for further analyses. First, cell edge contours are extracted and normal velocities are determined. Second, we measured cytoplasm flow via optical flow detection. Figure 1 shows contours for the front (3) and back (4) of a satellite. A more detailed analysis is in progress [5]. A particular phenomenon here is the period doubling of oscillations at the front, which was studied in detail in 6 satellites. Also, the movement speed of the membrane is approximately twice as high in the uroid region as in the front. Additionally, waves travel along the backside, whereas the front shows a uniform back- and forth movement. These findings were similar for more than 10 satellites.

3. F-ACTIN DISTRIBUTION

Physiologically, the cytoskeleton is responsible for the observed oscillations, especially the contractile apparatus of

.

BICT 2015, December 03-05, New York City, United States
Copyright © 2016 ICST
DOI 10.4108/eai.3-12-2015.2262491

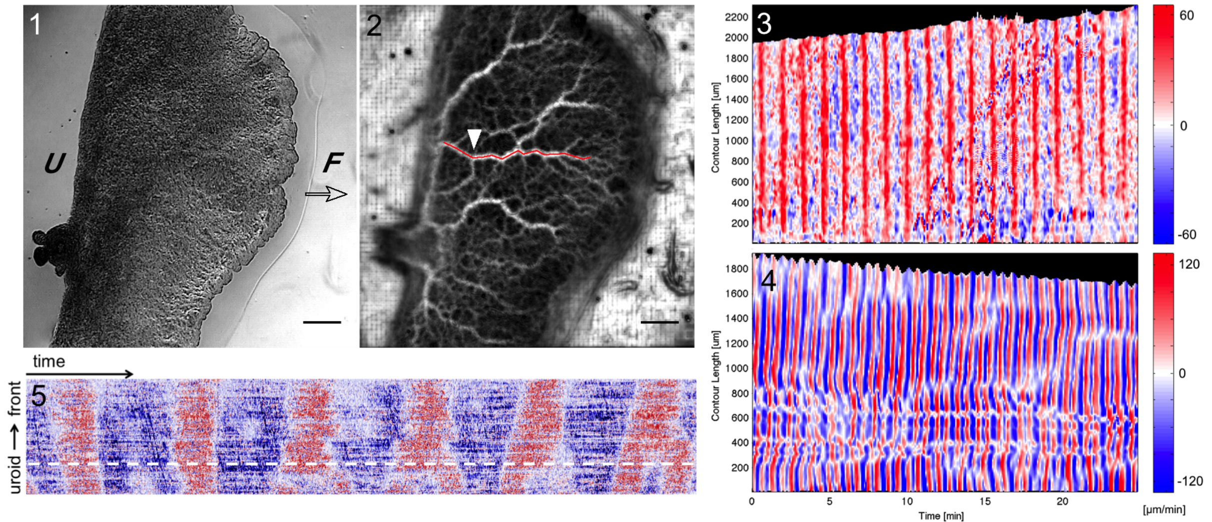

Figure 1: 1) Bright field image of a satellite. Movement is directed towards the right (arrow). U = uroid, F = front. 2) Image that is the sum of all slices in the stack, i.e. a sum of the time series resulting from optical flow analysis. Bright pixels mean large vectors, i.e. high flow velocities. Scale bar in 1) and 2) is 200 μm. White arrowhead denotes the location on the vein where two different oscillation patterns merge (dashed white line in 5). 3) Contour movement of the front of a satellite. Red = forward movement, blue = backward movement. 4) Contour movement of the uroid. The colour bar gives the velocities. 5) Kymograph of the flow velocities of a vein (red trace in 2) for the first ten minutes of the sequence. Lower left corner is the uroid region (left side of the vein). Red = flow towards the front, blue = flow towards the uroid.

the slime mould's periphery. Therefore, we rendered it visible via fluorescently labelled F-actin and investigated its distribution [5]. In the front, long, organized fibres are lacking, whereas the F-actin cytoskeleton in the back is very pronounced. Bright spots near the front seem to be bundles of F-actin, which could play a part in connecting the satellite to the substrate.

4. AN INTEGRATED PICTURE

Although the focus of this study lies on satellites, mainly because of their regular motion and convenient size, we believe that our findings apply to larger plasmodial networks as well. In fact, all investigations described above were also performed on larger networks. A detailed assessment is underway [5]. For example, the investigation of F-actin in large networks of *P. polycephalum* showed elaborate F-actin helices around the veins which enable the vigorous contractions that are the source of the shuttle streaming. We found similar, albeit smaller F-actin structures in the uroid and along the flanks of satellites which leads us to believe that they fulfil the same purpose.

We assume from the contour analysis that the uroid of the satellite acts as a pacemaker and creates a flow of endoplasm towards the front. In satellites with period doubling, an addition of the local oscillations leads to beating. Thus, the frequency in the front is lower. Kymographs of the flow velocities within the veins show that the addition of oscillations takes place close to the back end of the satellite, where the oscillators are located. The front is pushed outwards in a passive way by the inflowing endoplasm. This can be corroborated by correlation analysis and the distribution of F-actin throughout the satellite. A well organized cytoskeleton in the back, which is the driving force of the oscillations, is juxtaposed to the less dense and less organized actin at the front. Interestingly, vertical TEM images of a satellites show no traces of veins in the front of a satellite. They seem to be created entirely by the flow of endoplasm, as they lack walls. Lateral flow can also be observed, with cytoplasm diffusing through the sides of veins. Still, the internal vein system is persisting when the satellites moves forward. This can be seen from figure 1.2.

Assessing the complex process of amoeboid locomotion from different angles leads to many isolated observations. We aim to combine these pieces into one big picture of the locomotion of *P. polycephalum.*

The investigation of amoeboid locomotion is not only of fundamental interest for cell biology. Understanding this type of movement, which requires no specific organs like cilia or flagella, is also of interest for technical applications: Mobile robots could be realised, based on the amoeboid locomotion scheme, which could be used to navigate difficult terrain. This has been done for *Amoeba proteus* [6]. Soft-bodied robots which mimic *P. polycephalum* have already been constructed [7, 8]. Thus, in addition to emulating the slime mould's complex behaviour on a chip, the technical implementation of its motional properties seems promising as well.

5. REFERENCES

[1] K. Alim, G. Amselem, F. Peudecerf, M.P. Brenner, and A. Pringle. Random network peristalsis in Physarum polycephalum organizes fluid flows across an individual. *PNAS*, 110, 2013.

[2] E. Bernitt, C. Oettmeier, and H. G. Döbereiner. Microplasmodium dynamics of Physarum polycephalum. *IFMBE Proceedings*, 31, 2010.

[3] A. Fessel, C. Oettmeier, E. Bernitt, N. C. Gauthier, and H.-G. Döbereiner. Physarum polycephalum percolation as a paradigm for topological phase transitions in transportation networks. *Physical Review Letters*, 109, 2012.

[4] T. Lämmermann and M. Sixt. Mechanical modes of 'amoeboid' cell migration. *Current Opinion in Cell Biology*, 21, 2009.

[5] C. Oettmeier, and H.-G. Döbereiner (*unpublished*).

[6] W. Alt, V. Böhm, T. Kaufhold, E. Lobutova, C. Resagk, D. Voges and K. Zimmermann. Theoretical and experimental investigations of amoeboid movement and first steps of technical realisation. *Notes on Numerical Fluid Mechanics and Multidisciplinary Design*, 119, 2012.

[7] T. Umedachi, R. Idei, T. Nakagaki, R. Kobayashi and A. Ishiguro Fluid-filled soft-bodied amoeboid robot inspired by plasmodium of true slime mold. *Advanced Robotics*, 26, 2012.

[8] T. Umedachi, R. Idei, K. Ito and A. Ishiguro True-slime-mould-inspired hydrostatically coupled oscillator system exhibiting versatile behaviours. *Bioinspiration & Biomimetics*, 8, 2013.