Coordinations of Intracellular Flow, Calcium Signal and Cellular Contraction in Migrating *Physarum*

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ABSTRACT

This work was presented at PhysNet 2015. In this work, we jointly measure the intracellular flow, traction stresses and free intracellular calcium level ([Ca^{2+}]i) in *physarum* microplasmodium during amoeboid locomotion. Our measurements relates, for the first time, the intracellular mass transportation, the forces applied on the substrate and the signal of [Ca^{2+}]i with high resolution in both time and space, enables a thorough study about the locomotive mechanism, shedding light on related biomimetic research. Two distinct migrating modes have been identified and studied.

Categories and Subject Descriptors

J.3 [**Life and Medical Sciences**]: Biology and genetics

Keywords

amoeboid locomotion, traction force microscopy, calcium signaling, cytoplasmic streaming

1. INTRODUCTION

Amoeboid migration plays an important role in many physilogical processes such as tissure renewal, wound healing and immune response. It is a complicated process involves the interplay among actin polymerization, substrate adhesion, cytoplasmic flow, etc. Even though researchers have been working on this subject for a long time, the coordination among intracellualr flow, generation of traction stresses, and the propagation of chemical signal is still poorly understood.

In this work, we focus on the amoeboid locomotion of small scale *physarum* fragments ($\sim 100\mu m$). At this length scale, the fragment performs directed locomition with active shuttle flow in a elongated shape while doesn't exhibit compilcated morphological changes [4], which become a perfect model to study the interplay of these auto-oscilated quantities.

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2. METHOD

Physarum Plasmodium were cultivated on argar gel. A small portion from the tip were cut and transfered to new agar plate. After tubular shape has been developed, Calcium Green-1 and Texas-Red dextran (Molecular Probes) were co-injected into the organism, enables a ratiometric measurement of local [Ca^{2+}]i. Then a smaller portion was excised and placed on PA gel, which was prepared according to the description [2]. The PA gel consists two layers: a base layer at bottom and a very thin layer ($\sim 10\mu m$) contained $0.5\mu m$ fluorescent beads on top.

Images were aquired in both bright and fluorescent channels. The acquisition period is about 15 seconds, allowing us to obtain a quasi-simultaneous qunatification, given that the typical period of *physarum* locomotion is about 100 seconds.

We ran Particle Image Velocimetry (PIV, [6]) algorithm on bright filed images to get velocity field of intracellular flow. The 3D gel deformationat at the surface was determined by using the 3D PIV technique. Then the whole deformation field were determined by solving elasto-static equations using the measured beads displacement as boundary conditions. After that, the traction stresses were computed by solving Hooke's law.

3. RESULT

Upon reaching an adequate size ($\sim 100\mu m$ across), the cells adapt to elongated shape with noticeable shuttle flow and perform a directed locomotion with stable periodicity [4]. The dominant feature of traction stress is pure contractile, with larger stress distributed along the cell boundary and directed inward. This has been hypothesized as a result of strong cortex tension directed out on the substrate [1]. We then remove the average cortical stress to further analyze the dynamic part of the traction stress.

Calcium is known to be an important messager for cellular contraction. There are many strong evidences in favor of the conclusion that calcium acts as an inhibitor for *physarum* [5]. We found that the high local concentration of [Ca^{2+}]i always coincides with local relaxation while a low [Ca^{2+}]i will induce the cellular contraction, which is consistent with most rencent studies.

We observe two distinct locomotive modes. In the first case, we see travelling waves of the flow and traction stress propagating from posterior to anterior along the centerline. We call this peristaltic mode. For peristaltic cells, their behaviors are similar to that reported in [4]. As for the other mode, we call it amphistaltic mode due to the fact that the rear and front contract and relax in an anti-phase

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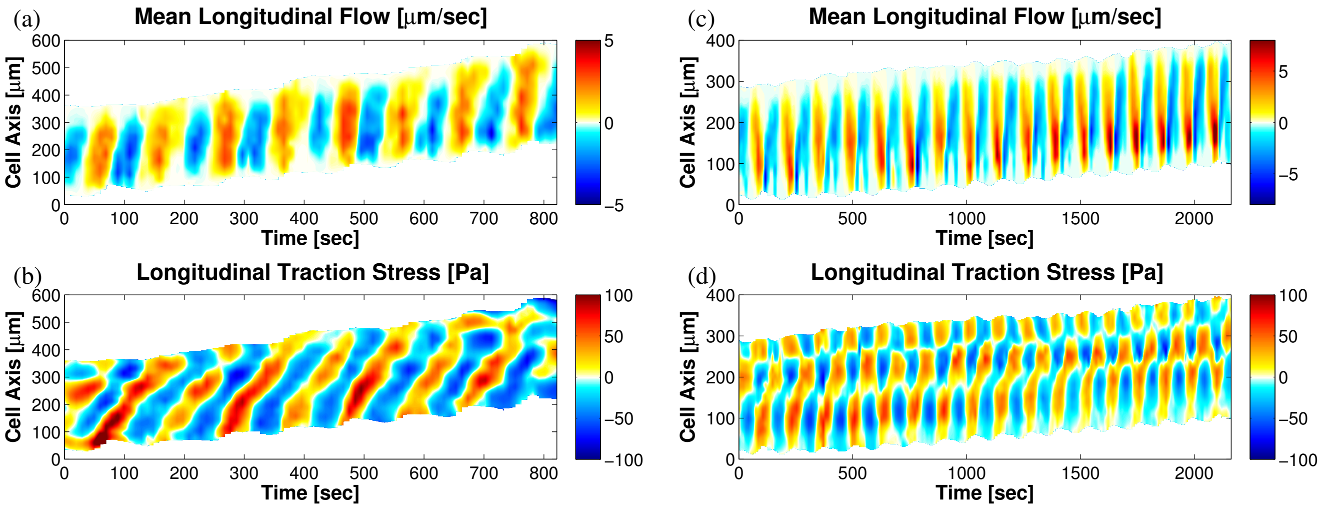

Figure 1: (a) Flow kymograph of *physarum* migrating using the peristaltic mode. (b)Traction stress (with average removed) kymograph of *physarum* migrating using the peristaltic mode. (c) Flow and kymograph of *physarum* migrating using the amphistaltic mode. (d) Traction stress (with average removed) kymograph of *physarum* migrating using the amphistaltic mode.

manner like a standing wave.

To better analyze the spatio-temporal evolution of flow and traction stress, we generate kymographs for them. In a kymograph, the measured longitudinal quantity is averaged along each cross section,

$$\overline{Q}(t) = \frac{\int_{\Omega_c} \vec{q}_f \cdot \hat{x}\, dy}{\int_{\Omega_c} dy}, \tag{1}$$

where Ω_c denotes the interior of the cell, x is the longitudinal coordinate coincides with the body axis, y is the coordinate orthogonal to the longitudinal axis, and $\hat{x}$ is unit vector oriented towards the anterior of the cell.

Figure 1 illustrates the experimental measurements of averaged longitudinal flow and traction stress for two modes. The periodic patterns are evident. Figure 1(a) shows a typical flow pattern of peristaltic mode, the forward and backward flow are all generated from the tail and propagate forward. Figure 1(b) shows the traction stress of the same experiment as Figure 1(a). After removing the average cortex stress, we also find clear travelling wave pattern. The behavior of peristaltic cells is discussed in detail in our previous work [3]. Figure 1(c) shows the kymograph of longitudinal flow of a cell using amphistaltic mode. The pattern is very different from peristaltic ones. The forward flow is originated from the head and propagates backward. Figure 1(d) is the kymograph of traction stress after removing the mean for the same experiment as Figure 1(c). Instead of a travelling wave, the amphistaltic cells using a standing wave which contracts and relaxes at the head and tail in an anti-phase manner. We found that the migration speed of peristaltic cells is significantly larger than amphistaltic ones, which are $0.1744 \pm 0.0399 \mu$m/sec and $0.0609 \pm 0.0294 \mu$m/sec respectively.

4. DISCUSSION

In this work, we simultaneously measure the intracellular flow, traction stresses and [Ca^{2+}]i. Our results reveal that the motility of *physarum* amoebae involves well-defined coordination of these quantities. Two dinstinct locomotive modes have been identified and studied. The significantly larger migration speed of peristaltic mode over amphistaltic mode may shed some lights on the design principles of biomemetic robot. In the future we will develop complete mathmatical model take aforementioned coordinations into consideration and apply pharmalogical treatment to the sample in order to acheive a further understanding of amoeboid locomotion.

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