Slime mold network reshaping induced by confinement

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Network dynamics is a subject of general interest, often hard to investigate because of its slow dynamics. *Physarum Polycephalum*, a true slime mould is an unicellular organism taking the shape of a network with complex self-organizing behaviors. Here, we explore its network emergence and directionality by suddenly confining it in a ring chambers. By using a structure tensor based image analysis method specifically adapted to our configuration, we have been able to derive quantities which capture these two dynamics. We show that confinement induces a reorganization of the network with a $10^4$ time scale, making its directionality align with the ring chamber main axis and that network emerges with two different time scales, one being compatible with network reorganization. Furthermore, we also observe that network dynamics for both emergence and reorganization conserve local features. Interestingly, these observations hint simultaneously towards flows driven network morphogenesis with no particular coordination at the organism scale.

*Physarum Polycephalum* is a multinucleated single cell organism taking the shape of an always adapting and exploring biological network. Despite its apparent simplicity, its potentially reversible life cycle made the slime mold a rich model for developmental biologists with research ranged into three topics: differentiation and development, cell cycle, and motility [1]. More recently it also became a subject of interest for the self-organizing behavior of its tubular network and the use of these behaviors to solve complex problems [2]. This problem solving ability which sometimes makes it being deemed as "intelligent", shows that it must have a mechanism to move information along its tubes. This communication which may take the form of chemical signal or nutrient concentration is insured by giant cytoplasm streaming referred as shuttle flows [3] which is the result of cross-sectional contractions organized in peristaltic waves [1]. This coordinated contractile activity has been extensively studied for various sizes and geometries [4][8], in presence or not of locomotion [9] but its relationship to the network self-organizing dynamics has never been understood. In particular, the huge difference in time scales makes the comparison difficult. Theoretically, network architecture has only be considered as static elements in oscillations model and shaping dynamics is never considered [10][12]. Experimentally, to our knowledge, entire network reorganization has never been characterized because of the difficulty to follow a multiplicity of observables on long times scales, moreover to extract meaningful information. The goal of this Letter is to show an experimental set-up and analysis method able to tackle this issue and obtain a first characterization of two different network dynamics: emergence and reorganization.

We consider here an experimental set-up where *Physarum polycephalum* is confined in ring-shaped chambers of controlled geometrical dimensions which has already been used to observe contractile oscillations patterns (see [8]). The ring perimeter $L$ (measured at the center) vary from 6.0 to 13.5 cm and the aspect ratio $L/e$, with $e$ the width of the ring is varying from 11 to 41 allowing us to consider a pseudo 1-D description of the specimen. To make ring shaped specimen, the plasmodium is first inseminated from previous cultivation on a petri box half filled with 2% phytagel gel. Multiple insemination points are disposed in an initial large circle and the plasmodium cover the area delimited by the circle. When the gel is covered by the plasmodium homogeneously, ring plastic walls are used to cut multiple ring shaped specimen concentrically. The petri box is then closed and sealed, and after a typical settling time of 15–30 min, the video recording starts using transmitted light microscopy. RGB images are taken every 4 or 6 seconds for films typically lasting 4 to 12 hours (see

![FIG. 1: Bright field microscopy images showing (a) network emergence and (b) network reorganization for ring confined Physarum. Raw images are taken at $t = 0$ s and $t = 20000$ s](image-url)
where diagonalised and we call \( \lambda \) the eigenvalues of the structure tensor with \( \lambda_M > \lambda_m \) and \( \alpha \) is the angle between the ellipse big axis direction and the local horizontal coordinate.

The local network directionality which will be considered is then an implicit function of \( \theta \). To avoid any borders issues, all quantities are derived in the maximum inscribed rectangle in the angular sector (see Fig.2).

To quantify networks emergence and directionality, we consider a python image analysis method (scikit-image library) relying on the derivation of structure tensors:

\[
J = \langle \nabla f, \nabla f^T \rangle_{\mathcal{N}(R)} = \begin{bmatrix}
\langle \partial_x f, \partial_x f \rangle_{\mathcal{N}(R)} & \langle \partial_x f, \partial_y f \rangle_{\mathcal{N}(R)} \\
\langle \partial_y f, \partial_x f \rangle_{\mathcal{N}(R)} & \langle \partial_y f, \partial_y f \rangle_{\mathcal{N}(R)}
\end{bmatrix}
\]

(1)

where \( f(x) \) is the intensity value at pixel position \( x \), \( \langle f,g \rangle_w = \int_{\mathbb{R}^2} f(x)g(x)\mathcal{N}(R)(x)dx \) and \( \mathcal{N}(R) \) is a gaussian window of size \( R \). \( J \) is then defined for every point of the initial image and convey information of its neighborhood defined by the window function, typically the angular sector with \( R = \delta s/2 \) in our case. \( J \) can then be diagonalised and we call \( \lambda_m, \lambda_M \) and \( e_m, e_M \) respectively the eigenvalues and eigenvectors of the structure tensor defined for each pixel.

Using these values, we define the local orientation \( \alpha \), the local coherency \( C \) and the local energy \( E \):

\[
\alpha = \frac{1}{2} \arctan \left( 2 \frac{\langle \partial_x f, \partial_y f \rangle_{\mathcal{N}(R)}}{\langle \partial_y f, \partial_y f \rangle_{\mathcal{N}(R)} - \langle \partial_x f, \partial_x f \rangle_{\mathcal{N}(R)}} \right),
\]

(2)

\[
C = \left| \frac{\lambda_M - \lambda_m}{\lambda_M + \lambda_m} \right| \quad C \in [0, 1]
\]

(3)

\[
E = \lambda_M + \lambda_m
\]

(4)

which are then averaged on the angular sector. The orientation \( \alpha \) then gives the mean local gradient direction relative to the angular sector local horizontal which makes angle \( \theta - \pi/2 \) with the initial image vertical. By considering its absolute value, it quantifies how close the angular sector main gradient direction is to the ring main axis (the orthoradial direction). \( \alpha = 0 \), respectively \( \alpha = \pi/2 \), correspond to veins aligned, respectively orthogonal with ring main axis. The coherency \( C \) is a measure of the local gradient anisotropy, which gives a rough estimate of the presence of veins. The energy \( E \) gives the local variations magnitude and is then directly related with veins emergence.

To quantify network emergence and reorganization, we divide the experiments in two types of initial conditions. The first kind corresponds to homogeneously filled ring chambers (see Fig.[a]) and is used to characterize network emergence while the second starts with an initial network with no particular direction (see Fig.[b]) and is then used to characterize network reorganization.

**Network emergence**

We consider the coherency weighted energy \( E_C = CE/\sum C \). Using this definition, the energy related to network veins are amplified comparatively to other local intensity variations (like blebs appearance).

We then display figure Fig.[c] the kymograph of \( E_C \) for the network emergence shown in Fig.[d]. We observe a global increase of the weighted energy for all the angular positions \( \theta \) capturing the network emergence. However, the pattern shows local features, with strong variations between the different angular positions. Particularly we can observe that a strong value of energy at one position is not communicated to its neighbors. Interestingly this means that the different angular sectors does not communicate between them to synchronize their dynamics.

We then consider 12 different rings for 8 different specimens. We demonstrate that the different rings experiments globally show network emergence by looking at the distribution of weighted energy divided by its initial mean value \( E_C(t = 0) \) where \( E_C(t) \) is \( \theta \) averaged.
value of $E_C$ at time $t$. Fig.3b shows unambiguously network emergence with a mean weighted energy at later times higher than for $t = 0$ s. Interestingly the distribution is also a lot more spread with weighted energy still close to zero, which means that no network has emerged locally, and weighted energy values which are ten times higher than the initial mean value.

To characterize network emergence dynamics, we show Fig.3c mean weighted energy dynamics for the ring experiment shown Fig.1a. We observe an exponential behavior with a time scale $T = 1.69 \times 10^4$ s which is then followed by a drop and then by an increase again of the mean weighted energy. These last features are the result of the appearance of blebs and veins meandering. To avoid any external effects in the dynamics characterization, we now only consider part of the experiments where the emergence dynamics is clearly visible. We call $t_0$ the initial time of these time windows.

Since the weighted energy $E_C$ is not synchronized, we avoid initial distribution effect by considering normalised weighted energy $E^*_C(\theta, t) = E_C(\theta, t)/E_C(\theta, t = 0)$ and we display Fig.3d the mean value $\overline{E_C}$ over time. Two emergence time scales are then observed: a slow one $T^s_E = 4.13 \pm 1.46 \times 10^4$ s which has to be understood as a lower bound since the evolution can also be described as linear and a fast one $T^f_E = 1.69 \pm 0.54 \times 10^4$ s. Surprisingly, these two time scales can not be related to specific visible morphological features and may then be an effect of internal dynamics (nutrients concentrations, cell life cycle or flows dynamics).

**Network reorganization**

We now are considering a second set of experiments where the plasmodium which originally spans the petri box has already formed a network. In this case, the initial conditions correspond to ring shaped network with no clear directionality. We use the $\alpha$ angle which quantify the local network directionality relatively to the ring main axis to characterize the network reorganization.

We show Fig.4a a kymograph showing the evolution of $\alpha$ over ring angular sector and over time for the ring experiment shown Fig.1b. We observe a convergence of $\alpha$ towards lower value then a second phase where $\alpha$ increases. If the first phase can clearly be related to a visual reorganization of the network, the second phase corresponds to the appearance of blebs and particularly
of vein meandering. While we could expect $\alpha$ to get to a stationary value, long term vein meandering increases it by inducing scale separation between really small-scaled curved features and global network directionality. Same as for weighted energy, we also observe Fig.4a that the local features are conserved along time despite appearing as locally strong variations suggesting that no communication exist between the different angular sectors at this time scale.

Same as previously, we now consider 7 ring experiments for 6 different specimens for which network reorganization is clearly visible. We show Fig.4b the $\alpha$ distribution at times $t = 0$ s and $t = 14000$ s to avoid meandering issue. We observe unambiguously that $\bar{\alpha}$ is lower at later time showing that network reorganization induces a network architecture more aligned with the ring main axis. The distribution is also less spread at later times showing the convergent feature of reorganization dynamics.

We now consider $\bar{\alpha}$ dynamics for the example shown Fig.1b. We observe Fig.4c the two stages discussed beforehand corresponding respectively to network reorganization and veins meandering. The first stage is well adjusted by an exponential fitting with a time scale $T = 1.03 \times 10^4$ s. To insure to get only reorganization dynamics, we now only consider time windows where network spatial reorganization are clearly visible and where blebbing and meandering does not occur. Like previously, we will call $t_0$ the initial time of these time windows.

We then systematically observe a converging dynamics which we fit with an exponential behavior. Since $\bar{\alpha}$ dynamics converges towards a value which is highly dependent on the ring width and on how reticulated is the network, a free constant value $C$ is added the exponential fit:

$$t \rightarrow A e^{t/T} + C$$

This gives three free parameters $A$, $T$ and $C$. We define $\Delta \bar{\alpha} = \bar{\alpha} - C$ the redefined mean network direction relative to this convergence value. We observe Fig.4d that despite a strong dispersion between the different experiments, we observe that $\Delta \bar{\alpha}$ follow an exponential behavior with a common time scale $T_{\alpha} = 0.93 \pm 0.32 \times 10^4$ s which corresponds to the mean value of the fitted time scales $T$. The error bars correspond to the standard deviation of the distribution of $T$. On some experiments, the second phase can also be observed where the directionality be-
gins to increase again showing the difficulty to obtain a completely unambiguous behavior. Interestingly the directionality time scale $T_\alpha$ is close to the weighted energy fast time scale $T'_E$ which suggests a common mechanism driving both dynamics.

**Discussion**

We considered here an image analysis method to obtain information on network dynamics of ring confined *Physarum Polycephalum*. This method relies on a tool called the structure tensor giving for each pixel the gradient information of its local neighborhood. Using quantities derived from this method, we were able to characterize for two sets of experiments *Physarum* network emergence and reorganization.

Firstly, we confirm the presence of network emergence from homogeneous plasmoidium and reorganization from random network correctly captured by the structure tensor image analysis method. Then we observe that both network emergence and reorganization conserve local structures are conserved along time which hints towards mechanisms which does not suppose communication or transport mechanism between the different positions of the organism. For network emergence dynamics, we observe two different trends: a fast exponential one with a time scale $T'_E = 1.69 \pm 0.54 \times 10^4$ s and a slower one giving a time scale $T_E = 4.13 \pm 1.46 \times 10^4$ s but can also be deemed as linear. The origin of this difference is still not understood. For network reorganization, we demonstrate that the directionality of the network converges towards the ring main axis showing the effect of geometrical constraints upon network dynamics. This convergence occurs on a time scale $T_\alpha = 0.93 \pm 0.32 \times 10^4$ s which is two orders of magnitude slower that contractile oscillations. These results hint towards the hypothesis that networks are shaped by the integrated flows over time following the idea that they open channels on the plasmoidium [13]. Indeed flows have to follow the ring geometrical constraints and while they can have transient variations on the time scale of the contractile oscillations, the big difference in time scales smeared them out.

We think that these results: the time scale of the order $10^4$ s and the confinement induced network directionality is completely general for macroscopic physarum size since no dependency on ring size has been clearly observed. Of course, other geometrical constraints less strict should be tested using light, humidity or nutriments to verify the generality of this claim. However we think of this result to be of interest for any experiment involving geometrical constraints on Physarum Polycephalum since it provides a way to control experimentally the network architecture. Intriguingly the $10^4$ s time scale observed is similar to the one observed by Rodiek and Hauser [14] for freely migrating microplasmodia to attain diffusive behavior. Below this time scale, migration tends to look like successive straight persistent motions. Hence this time scale may be the minimum time necessary for the organism to adapt its network and change its direction.

**ACKNOWLEDGMENTS**

We acknowledge financial support from French National Research Agency Grants ANR-17-CE02-0019-01-421 SMARTCELL and CNRS MITI "Mission pour les initiatives transverses et interdisciplinaires (Reference: BioRes). We also thank the MSC laboratory mechanical workshop for their technical contributions to this project.

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