Multi-scale interactions in *Dictyostelium discoideum* aggregation

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**Abstract**

Cellular aggregation is essential for a wide range of phenomena in developmental biology, and a crucial event in the life-cycle of *Dictyostelium discoideum*. The current manuscript presents an analysis of multi-scale interactions involved in *D. discoideum* aggregation and non-aggregation events. The multi-scale fractal dimensions of a sequence of microscope images were used to estimate changing structure at different spatial scales. Three regions showing aggregation and three showing non-aggregation were considered. The results showed that both aggregation and non-aggregation regions were strongly multi-fractal. Analyses of the over-time relationships among nine scales of the generalized dimension, \(D(q)\), were conducted using vector autoregression and vector error-correction models. Both types of regions showed evidence that across-scale interactions serve to maintain the equilibrium of the system. Aggregation and non-aggregation regions also showed different patterns of effects of individual scales on other scales. Specifically, aggregation regions showed greater effects of both the smallest and largest scales on the smaller scale structures. The results suggest that multi-scale interactions are responsible for maintaining and altering the cellular structures during aggregation.

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1. Introduction

Cell aggregation is an important precursor for a host of events in developmental biology, including cell-fate decisions [1] and differentiation [2]. Aggregation requires the coordinated activity of many levels of cell behavior, such as intra- and intercellular “signaling” [3], cell motility [4], and the adhesion of adjoining cell membranes [5]. As such, aggregation is a macro-level phenomenon that is supported by the activity of many levels within the system.

Aggregation is also a fundamental phenomenon in the study of complex physical materials, such as those found in cloud formation, electro- and magneto-rheological fluids, colloidal gases and gels, and polymers [6–9]. In these domains, aggregation often occurs at multiple scales. For example, in complex fluids, such as colloidal suspensions, individual particles coalesce to form mesostructures, which in turn link together to form clusters. These clusters can ultimately form a macrostructure that spans the entire sample. An important insight from this work is that the properties of the fluid in which the aggregates have formed are not reducible to the micro-properties of the particles. Rather, the aggregates themselves exert influence through mechanical interactions and through effects on the hydrodynamics [10–12].
The current study addresses cellular aggregation in Dictyostelium discoideum from the perspective of multi-scale structures and their interactions. Causal physical structures in the Dictyostelium system exist at many scales [13,14]. Some of these structures are well known, such as cell membranes and cAMP gradients [15,16]. Others are less obvious, such as the adhesion complexes of pseudopods [17], but all must have measurable effects on the system, if they affect aggregation. Importantly, recent work shows that mechanical forces are not just the distal outcome of signaling processes that control cellular behavior. Rather, mechanical forces feed back into these signaling pathways through processes such as protein stretching [1] and stretch-activated calcium channels [18]. Thus, the activity generated by multi-scale processes within the system, also affect those self-same processes.

Because it is not known how many structures are in play during cell aggregation or at what scales they operate, our strategy is to measure the system across a wide range of scales. Each measurement quantifies the structures in that region of scales in a way that relates to their thermodynamic and hydrodynamic properties. We then use these measures to predict the changing structures at multiple scales. Our goal here is to show how interactions across scales drive aggregation (and non-aggregation) in Dictyostelium. The current approach treats the structured matter that constitutes the collection of cells as a multifractal field and proposes that interactions within that field are causally related to the macroscopic behavior of the system. A complementary approach is to extrapolate models from known processes at multiple scales (e.g., individual cells, tissues) and eventually model their interplay. See Refs. [19,20] for excellent examples of this latter approach.

For relatively simple, homogeneous media with a single type of particle structure (or very few structures), it is possible to determine hydrodynamic and thermodynamic properties analytically, given the fractal dimension [21]. For more complex media with heterogeneous structures, it is necessary to empirically determine their fractal dimensions, and use those measures as predictors of the behavior of the system, relying on their known links to hydro- and thermodynamics [10]. Given the very complex nature of cellular structures, we estimated the fractal dimensions across a range of different magnitudes (via a multifractal formalism) and used those estimates as measures of structure at multiple scales. We expected that structures at each scale would potentially have causal effects on all other scales, and that regions in which aggregation occurs would have different patterns of interaction compared to regions in which aggregation did not ultimately occur.

Carrillo et al. [22] utilized a related approach in their investigation of two different types of rheological dispersions, an electro-rheological fluid and a magneto-rheological fluid. Each dispersion began with a single type of particle (Bi$_4$Ti$_3$O$_{12}$ and Fe$_3$O$_4$, respectively) suspended in silicone oil at low concentration (volume fraction $<0.05$). They filmed the process of pattern formation as a field (electric or magnetic) was applied. From the resulting images, Carrillo et al. calculated the mass fractal dimension, $D$. Their results showed three distinct scaling regions, indicative of aggregations with three different characteristic scales. The first of these aggregates to emerge (i.e., the smallest scale) is driven primarily by dipolar interactions. The second–order aggregate (i.e., the middle scale) depends on both liquid viscosity and mechanical interactions among the first–order clusters. The formation of the third-order aggregate is slower, because of the shorter interaction time among second–order clusters and effects of viscosity.

Like Carrillo et al., we calculate the fractal dimension, $D$, from images taken during aggregation. We sampled six regions from a 541 frame (eight-bit, 672 × 512 pixel) recording of a Dictyostelium aggregation event. Each sampled region was 220 × 230 pixels. We selected three of these regions because they became aggregation sites. The remaining three were selected because they showed considerable activity, but aggregates did not form there. We performed multi-fractal analysis on each frame for each of the six regions. Multifractal analysis estimates the fractal dimension, $D$, across a spectrum of scales. For mono-fractals, the estimated values of $D$ will vary little across scales. For multi-fractals, $D$ will decrease monotonically with an increase in scales. The value of $D$ within the spectrum provides a measure of the local fractal dimension for that scaling region. The details of the analysis are presented below, but here we note that the fractal dimension of structures at any particular scale relates to its energetic properties. For example, the degree to which a structure interacts with neighboring structures depends on its fractal dimension; greater fractal dimension increases the strength of interactions [10,11]. Similarly, the degree to which particles can move (e.g., via diffusion) near the structure is determined by the fractal dimension of the structure. The fractal dimension of a structure also affects the osmolar pressure within the bounded region in which the structure sits [21].

It is perhaps worth noting here that unlike Carrillo et al.’s rheological fluids in which there is initially very little structure (i.e., the particles are homogeneously distributed at first), the Dictyostelium images reveal considerable structure from the start. There is obviously structure within the cells themselves, but also among the cells.

To model the effects of $D$ at each scale on future states of all other scales, we adopted a standard set of methods from econometrics for addressing systems in which all variables are treated as potentially endogenous and mutually affecting (i.e., each variable may affect each other variable) [23,24]. This family of methods, called vector autoregression and vector error-correction models, allows us to treat the fractal dimension at each scale as both a predictor (at a particular lag) and an outcome. We show that all scales have significant effects on the system. We also show that none of the scales functions as an exogenous variable. Finally, we show that aggregation and non-aggregation sites have different patterns of across–scale effects.

In the next sections, we describe the microscopy used to obtain the images during Dictyostelium aggregation and then review the multifractal analysis used to quantify the structures in those images. Finally, we explain the vector autoregression/error correction methods used to model the system-level effects and across-scale interactions.
2. Material and methods

2.1. Cell line and microscopy

Ax2 (wild-type) cells were grown at room temperature in HL5 medium (5 g proteose peptone #2, (Difco, Detroit, MI) 5 g thione E, (Becton Dickinson, Cockeysville, MD) 10 g glucose, 5 g yeast extract, 0.35 g Na2HPO4, 0.35 g KH2PO4, 0.1 mg/ml ampicillin, 0.1 mg/ml dihydrostreptomycin sulfate, pH 6.5) in plastic petri dishes. Cells expressing GFP were made by transfecting Ax2 cells with the PTX-GFP vector which expresses GFP constitutively from an actin 15 promoter. Cells were transfected and selected for resistance to G418 as described previously [25].

Cells were harvested by trituration, centrifuged at 1000 rpm for 5 min and washed twice with MCPB buffer (10 mM Na2HPO4, 10 mM KH2PO4, 2 mM MgCl2, 0.2 mM CaCl2 (pH 6.5)). The cells were then resuspended in MCPB at a concentration of 2.5 × 106 cells/mL. A mixture of 10% fluorescent (GFP-expressing) and 90% wild-type containing a total of 5 × 106 cells was then plated on a 30 mm glass-bottom Petri dish (Willco Wells, Amsterdam, The Netherlands) and allowed to attach for 30 min. During this incubation, 2 mL of 1.5% agarose in MCPB buffer was poured into a second 30 mm glass-bottom Petri dish and allowed to solidify. After the 30 min incubation, the agarose is removed from the dish and placed directly on top of the buffer in the dish with the cells. The buffer was removed allowing the agarose sheet to settle gently onto the cells.

2.2. Computing multifractal dimension of images

2.2.1. Monofractal box-counting dimensions: Binary and grayscale

We used an extension of a standard box counting algorithm to calculate the fractal dimension of images [26]. The box-counting dimension \( D_{\text{box}} \) is a metric of how pixel intensity is distributed across measurement scales. The box-counting algorithm imposes a series of grids onto the image. Each grid is composed of square boxes with length \( L \). For a binary image composed only of foreground and background, each box must either contain a part of the foreground or contain only a part of the background. For binary images, dimension \( D_{\text{box}} \) is calculated as:

\[
D_{\text{box}} = \lim_{L \to 0} \frac{\log N(L)}{\log L}, \tag{1}
\]

where \( N(L) \) is the number of boxes with length \( L \) containing a part of the foreground.

For grayscale images, one can make a more subtle distinction than that between foreground and background. The advantage of this grayscale analysis is that it makes use of the rich information in non-binary images. The grayscale box-counting algorithm computes relative intensity \( R \) for each box \( x, y \) of length \( L \):

\[
R(x, y, L) = I_{\text{Max}}(x, y, L) - I_{\text{Min}}(x, y, L), \tag{2}
\]

where \( I_{\text{Max}}(x, y, L) \) and \( I_{\text{Min}}(x, y, L) \) are the maximum and minimum intensities, respectively, of pixels within each box \( x, y \). Then, \( R(x, y) \) is used to compute a volume \( V(x, y, L) \) as follows:

\[
V(x, y, L) = R(x, y, L) \times L^2, \tag{3}
\]

where \( L \) is the length of the boxes in the grid. Subsequently, the formula for the grayscale box-counting dimension \( D_{\text{GrayBox}} \) replaces \( N(L) \) from Eq. (1) with the sum of these volumes for a given length \( L \):

\[
D_{\text{GrayBox}} = \lim_{L \to 0} \frac{\log \sum V(L)}{\log L}. \tag{4}
\]

We use grayscale rather than binary box-counting methods throughout. FracLac for ImageJ was used to compute the volumes, \( V \), at each box length \( L \).

2.2.2. Multifractal box-counting dimension

The standard grayscale box-counting dimension \( D_{\text{GrayBox}} \) is only one dimension in an infinite spectrum of dimensions. The implicit assumption of the standard (i.e., monofractal) case is that volumes of all magnitudes respect the same fractal scaling. It is possible to test for differences in fractal scaling for volumes of different magnitudes by manipulating a moment \( q \). Multifractality is a dependence of box-counting dimension \( D(q) \) on the moment \( q \). The moment \( q \) is applied as an exponent on the volume \( V(x, y, L) \) of each box \( x, y \) of length \( L \). Hence:

\[
D_{\text{GrayBox}}(q) = \frac{1}{q - 1} \lim_{L \to 0} \left( \log \sum (V(L)^q) / \log L \right). \tag{5}
\]

The \( q \) parameter functionally magnifies different regions of the distribution of volumes. As \( q \) increases, volumes of larger magnitude are emphasized at the expense of smaller volumes. As \( q \) decreases, volumes of smaller magnitude are emphasized at the expense of larger volumes.
Images that are multifractal exhibit a particular set of relationships. For multifractal images, the fractal dimension $D_q$ decreases monotonically as $q$ increases. Similarly, the singularity spectrum, $f(\alpha)$, will be a smooth, single-peaked function, where $f(q)$ is the Hausdorff dimension [27–29],

$$\mu_i(q, L) = [V_i(L)]^q \sum_j [V_j(L)]^q$$

$$f(q) = \lim_{L \to 0} \sum_i \mu_i(q, L) \log [\mu_i(q, L)] / \log L,$$

and $\alpha(q)$ is the Holder exponent,

$$\alpha(q) = \lim_{L \to 0} \sum_i \mu_i(q, L) \log [V_i(q, L)] / \log L.$$

### 3. Results and discussion

#### 3.1. Multifractal analysis

We defined six regions of interest for each of the 541 images in the sequence. A single frame, the ninth in the sequence, was unusable and was dropped from the remaining analyses. Examples of each of the six regions are shown in Fig. 1. As can be seen in the figure, the images appear quite similar early on. However, near the end of the recording, the aggregation sites are well defined.

For each of the 540 frames, for each region of interest, we performed gray-scale box counting from 12 different starting locations with FracIc for ImageJ. We used 20 box sizes, with $L$ ranging from 2 to 138 pixels for each of the 12 starting locations. We computed the multifractal analysis described above on the data obtained from each starting location for $-4 \leq q \leq 4$, incrementing by 0.5.

Following the recommendations of Zamir [30], we examined the linear fit of the regression used to estimate the Hausdorff dimension, $f(q)$, and Holder exponent, $\alpha(q)$, for each level of $q$ for each starting location. Zamir noted that using the method of Chhabra and Jensen, one could mistakenly infer multifractality in empirical data if the relationships used to estimate $f(q)$ and $\alpha(q)$ (see Eqs. (7) and (8) above) were not linear. Inspection of the current results showed that the nonlinearity in the relationship used to estimate $f(q)$ was always the more sensitive of the two for these samples. If the linear fit used to estimate $f(q)$ had an $R^2 < 0.95$, we tested the fit of the quadratic by adding $(\log L)^2$ to the model. If the $t$-value for the coefficient of $(\log L)^2$ was greater than 2, the multifractal estimates from that $q$ for that starting location were not considered further. Thus, all the calculations are computed on data for which a reasonable linear relationship for $f(q)$ was obtained. The multifractal measures described above were computed for each starting location (that met the linear $f(q)$ criteria just described); we averaged over these starting locations to obtain the estimate of each measure for each of the six regions across the 540 frames in the sequence separately.

Fig. 2 shows the $f(\alpha)$ functions for various frames across the 540 frame sequence for each of the six regions of interest. Each panel shows the results from a single location with a separate curve for each of 11 frames, spaced 50 frames apart (e.g., frame 1, frame 51, etc.). As can be seen in the figure, the prediction of a single-peaked function was met; indeed it was met for all regions for all frames.

Fig. 3 shows the $D(q)$ functions for each of the six regions. Each panel shows the results for a different region, with a separate curve for 11 different frames within the 540 frame sequence. As can be seen in the figure, all the $D(q)$ functions decrease monotonically.

Fig. 4 shows $D(q)$ functions for all 540 frames for each location (in separate panels). Each curve shows a different level of $q$, from most negative, $-4$, to most positive, 3.5. Because $q$ is a continuous parameter, it is necessary to select some set of $q$ values. We chose to use nine levels of $q$, from $-4$ to 3.5. We eliminated the largest scale, 4, because a relatively large percentage of the 6480 samples (12 starting locations × 540 frames) for each of the six regions of interest did not meet the criteria discussed above, $M = 10\%, SE = 0.024$. For the remaining levels of $q$, very few were eliminated, $M = 0.001\%, SE = 0.0007$.

Regions 1–3 ultimately exhibited cell aggregation; regions 4–6 did not. A casual comparison of the time series of $D(q)$ for the aggregating and non-aggregating sites shows some potentially interesting differences. For example, the non-aggregating sites show a decrease in $D$ for the large positive and large negative scales. The aggregating sites show an increase in the negative scales, but the picture at the positive scales is less clear.

#### 3.2. Vector models

##### 3.2.1. Co-integration

We propose that the structures at different scales are causally and reciprocally affecting each other, and that these effects both maintain the system’s cohesiveness and, ultimately, drive it to a new organization. To test this set of hypotheses, we
treated the $D(q)$ measures over the 540 frames as a multivariate time series (for each location separately) using vector autoregression and vector error-correction models [31].

Vector autoregression (VAR) models were developed in econometrics to quantify the mutual effects among concurrent variables over time, $t; t = 1, 2, \ldots, T$. Rather than forcing each variable into the analysis as either predictor or outcome, VAR models treat each variable as the outcome of all endogenous variables at a lag of 1 minimally, although longer lags can also be used. For any system of $m$ endogenous variables, VAR produces a system of $m$ regression equations. The endogenous predictors in each equation are lagged values of all endogenous variables; exogenous variables may also be included. Assuming a lag-2 system, a VAR model would have the general form:

$$w_t = \Pi_1 w_{t-1} + \Pi_2 w_{t-2} + C + \varepsilon(t)$$

where $w_t$ is the $m$-dimensional vector comprising the endogenous variables, $\Pi_1$ is an $m \times m$ matrix of coefficients representing the effect of lag-1 values on $w_t$, $\Pi_2$ is a matrix of coefficients representing the effect of lag-2 values on $w_t$, $C$ is a vector of intercept terms, and $\varepsilon(t)$ is a vector of Gaussian error terms.

Any VAR model (with a lag of 2 more) can also be written as a vector error-correction model (VECM), which allows us to directly address a number of interesting possibilities, particularly whether the vector of endogenous variables is co-integrated. Co-integration entails that the variables are in a stable relationship with one another, such that perturbations are “corrected” (i.e., returned to equilibrium) by the system. Co-integration predicts that when a variable steps out of its

Fig. 1. Images from the sequence. Examples of images from various frames in the sequence for each of the six regions. Images for the three aggregation regions are shown in the bottom rows; the non-aggregation regions are in the top rows. A scale bar shows the approximate size of each region in $\mu$m.
equilibrium relationship, equilibrium is restored by some set (or potentially all) of the variables in the system. The simple VAR model above can be written as a VECM as follows:

$$\Delta w_t = \Phi \Delta w_{t-1} + P(Bw_{t-1}) + C + \epsilon(t)$$  (10)

where $\Delta w_t$ is the vector of first-order differences, $\Phi$ is an $m \times m$ matrix of coefficients representing the effect of lag-1 differences on $\Delta w_t$, $P$ is an $m \times r$ matrix of adjustment weights representing the effect of the error-correction terms, $B$ is an $m \times r$ matrix of coefficients representing the contribution of each variable in the vector $w_{t-1}$ to the error-correction term (the coefficients in one row are set to 1 to identify the model). The co-integration rank, $r$, can be roughly defined as the number of error-correction terms required for stability.
To determine the co-integration rank, we used a method developed by Johansen and colleagues [32]. Using the VECM representation, the first step is to regress the differences $\Delta w_t$ and the values $w_{t-k}$ on all lagged differences $\Delta w_t, \Delta w_{t-1}, \ldots, \Delta w_{t-k+1}$. The vector of residuals in each set of regressions, $R_{0,t}, R_{k,t}$, respectively, allows computation of four $m \times m$ matrices:

$$S_{ij} = \frac{1}{T} \sum_{t=1}^{T} R_{it} R_{jt}', \quad \text{for } i, j = 0, k$$

where $T$ is the total observations for all endogenous variables. Next, it is possible to derive $m$ eigenvalues $\lambda_1, \lambda_2, \ldots, \lambda_m$ from the polynomial:

$$|\lambda_{S_{kk}} - S_{00}S_{0k}^{-1}S_{0k}| = 0$$

where the first $r$ corresponding eigenvectors constitute $\hat{\beta}$ the maximum likelihood estimator (MLE) for $\beta$. Relatedly,

$$\alpha = S_{0k}\beta$$

provides the MLE for $\alpha$. As for determining $r$, there are two test statistics, the trace and the maximum eigenvalue statistic ($LR_{max}$):

$$\text{Trace} = -T \sum_{i=r+1}^{m} \ln(1 - \lambda_i)$$

$$LR_{max} = -T \ln(1 - \lambda_{r+1})$$

each of which test the hypothesis that, for each possible number of cointegrating vectors $0 < r < m - 1$, the subsequent eigenvalue is not significantly different from zero. When the statistics in Eq. (14) exceed the 5% critical values in their distributions [33], the next eigenvalue is significantly different from zero, and the corresponding eigenvector must be included among the cointegration vectors composing $\hat{\beta}$ [23].

We used the Johansen procedure with the $LR_{max}$ Statistic to evaluate the number of co-integration relations among the 9 $D(q)$ variables for each location separately. Table 1 shows the results of the co-integration rank tests. For each of the six
regions, the number of significant co-integration relations (i.e., co-integration rank) was either 5 or 6, $\alpha = 0.05$. These results provide evidence for the hypothesis that the $D(q)$ across scales form a self-regulating system. There is reasonable agreement across the regions in terms of the number of co-integration relations. The small differences may be due to the fact that the regions are unlikely to be perfectly homogeneous in terms of their initial conditions, rates of change, or final states.

$$\begin{bmatrix}
\Delta D_{94,t} \\
\Delta D_{93,t} \\
\vdots \\
\Delta D_{92.5,t} \\
\Delta D_{93.5,t}
\end{bmatrix} =
\begin{bmatrix}
\phi_{11} & \cdots & \phi_{19} \\
\phi_{21} & \cdots & \phi_{29} \\
\vdots & & \vdots \\
\phi_{81} & \cdots & \phi_{89} \\
\phi_{91} & \cdots & \phi_{99}
\end{bmatrix}
\begin{bmatrix}
\Delta D_{94,t-1} \\
\Delta D_{93,t-1} \\
\vdots \\
\Delta D_{92.5,t-1} \\
\Delta D_{93.5,t-1}
\end{bmatrix} +
\begin{bmatrix}
\rho_{11} & \cdots & \rho_{19} \\
\rho_{21} & \cdots & \rho_{29} \\
\vdots & & \vdots \\
\rho_{81} & \cdots & \rho_{89} \\
\rho_{91} & \cdots & \rho_{99}
\end{bmatrix}
\begin{bmatrix}
D_{94,t-1} - \beta_1 D_{93,t-1} - \cdots - \beta_{18} D_{92.5,t-1} - \beta_{19} D_{93.5,t-1} \\
D_{94,t-1} - \beta_2 D_{93,t-1} - \cdots - \beta_{18} D_{92.5,t-1} - \beta_{19} D_{93.5,t-1} \\
\vdots \\
D_{94,t-1} - \beta_9 D_{93,t-1} - \cdots - \beta_{18} D_{92.5,t-1} - \beta_{19} D_{93.5,t-1} \\
D_{94,t-1} - \beta_1 D_{93,t-1} - \cdots - \beta_{18} D_{92.5,t-1} - \beta_{19} D_{93.5,t-1}
\end{bmatrix}.$$  \( (15) \)

Eq. (15) shows the vector error-correction model for the full system, including six co-integration relations, with the ellipses indicating terms not shown but implied by the sequence within the vector. Two additional vectors, one of intercept terms and the other of error terms, are not shown. Change in each of the nine levels of $D$, where $n$ and $p$ indicate negative and positive respectively (e.g., $\Delta D_{94,t}$ is change in $D$ at time $t$ when $q = -4$), is modeled by two sets of terms. The matrix on the left-most side contains the coefficients, $\phi$, for previous, $t - 1$, changes in each of the levels of $D$. The six error-correction terms are in the right-most vector. The matrix of weights, $\rho$, controls the adjustment from each error-correction term to change in $D$. Each error-correction term in the vector is a linear relationship amongst the nine levels of $D$.

### 3.2.2. Tests of eliminability and weak exogeneity

A number of interesting hypotheses can be directly addressed, given this representation of the system. First, we can test whether each of the nine scales under consideration is predictively involved in the regulation of the system [24]. To address this issue, we systematically set all the $\beta$ to zero for a single scale for all co-integration relations simultaneously. The resulting change in log-likelihood ($-2LL$) of the model provides a test of whether that scale contributes to maintaining the system’s equilibrium. We performed this test for each of the nine scales separately, for each of the six locations. (In order to specify the model, we set $\beta = 1$ for the first variable entered. Thus, to test the effect of this scale we entered the variables in the opposite order.) Table 2 shows the results for this analysis. As can be seen in the table, eliminating any of the scales from the co-integration relations significantly decreases the fit of the model. Each of the scales appears to be contributing to the self-regulation of the system.

A second issue concerns the status of each scale with regards to the system. Variables that are weakly exogenous to a system have their dynamics independently determined by other forces at the current time step. Endogenous variables have their dynamics affected by the system itself. It is possible that some of the scales we have measured are unrelated to the current activity of the system. For example, one might suggest that the activities of individual cells are driven solely by the local distribution of cAMP, and that other scales in the system have no concurrent causal relation to their changing shape and position. To test whether any particular scale is weakly exogenous, we set all the $p$ to zero for that scale. The change in the $-2LL$ of the model provides a test of whether considering this scale as weakly exogenous significantly decreases the fit of the model. We tested whether each scale was weakly exogenous for each of the six locations separately. Table 3 shows the results of this analysis. As can be seen in the table, none of the variables appears to function exogenously; all variables are endogenous.
Table 2
Tests of scales contributing to equilibrium correction.

<table>
<thead>
<tr>
<th>Scales ($D_q$)</th>
<th>Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$D_{n4}$</td>
<td>150.95</td>
</tr>
<tr>
<td>$D_{n3}$</td>
<td>175.30</td>
</tr>
<tr>
<td>$D_{n2}$</td>
<td>177.38</td>
</tr>
<tr>
<td>$D_{n1}$</td>
<td>123.94</td>
</tr>
<tr>
<td>$D_0$</td>
<td>62.71</td>
</tr>
<tr>
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</tr>
<tr>
<td>$D_{p1}$</td>
<td>20.96</td>
</tr>
<tr>
<td>$D_{p2}$</td>
<td>13.97</td>
</tr>
</tbody>
</table>

Note: The entries in the table show the change in the $-2LL$ test statistic when each scale's contribution to the error-correction terms is removed. The critical value of the $\chi^2$ distribution is 14.07, $df = 7$, $\alpha = 0.05$.

Table 3
Tests of weak exogeneity for each scale.

<table>
<thead>
<tr>
<th>Scales ($D_q$)</th>
<th>Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$D_{n4}$</td>
<td>109.26</td>
</tr>
<tr>
<td>$D_{n3}$</td>
<td>124.92</td>
</tr>
<tr>
<td>$D_{n2}$</td>
<td>145.38</td>
</tr>
<tr>
<td>$D_{n1}$</td>
<td>176.50</td>
</tr>
<tr>
<td>$D_0$</td>
<td>193.46</td>
</tr>
<tr>
<td>$D_{p0}$</td>
<td>194.14</td>
</tr>
<tr>
<td>$D_{p1}$</td>
<td>191.61</td>
</tr>
<tr>
<td>$D_{p2}$</td>
<td>181.43</td>
</tr>
</tbody>
</table>

Note: The entries in the table show the change in $-2LL$ test statistic for removing the effects of the error-correction terms on each scale. The critical value of the $\chi^2$ distribution is 11.07, $df = 5$, $\alpha = 0.05$.

3.2.3. Impulse response functions

The preceding analyses have focused on relationships between each scale and the rest of the system (i.e., whether a scale contributed to equilibrium adjustments, and whether a scale was exogenous to the system). In these final two sections, we examine the unique effects of each scale on future states of other scales in the system using impulse response functions (IRFs) and forecast error variance decomposition (FEVD). These related methods are powerful means of studying the behavior of recursive dynamic systems, such as the vector error-correction model. The nonlinearities created by the recursive nature of such models disallow the direct interpretation of the coefficients. IRFs show how perturbations at one scale affect the future behavior at (potentially) all scales. The vector error-correction model for each region is first transformed into a vector autoregression model, and Cholesky decomposition is used to orthogonalize the effects of each scale. Unlike the analyses presented above, the IRF results depend upon the order in which the variables are entered. The effects of each variable are conditioned by other effects already in the model on that time step. Because the $q$ parameter provides a naturally ordered dimension, we considered two orderings for the IRF analyses: from the smallest ($-4$) scale to the largest (3.5) scale and from the largest to the smallest scale.

Fig. 5 shows the impulse response functions for effects of the smallest scale. Each panel shows the response of all nine scales (indexed by the variable “qresp”) to a 1 standard deviation innovation or “shock” from the smallest scale (the shock occurs only on the first time step and then is off). Within each panel, the first time step shows the contemporaneous effect of the shock; all subsequent time steps show effects of the shock as it propagates through the system.

The left-side columns show locations 1 through 3, locations at which aggregation occurred. The right side shows locations 4 through 6, where aggregation did not occur. The top row shows the results for the impulse response analysis when the smallest scale is entered first, the bottom row shows the results for when the largest scale is entered first. The columns show the result for different locations.

When the smallest scales are entered first (upper row), all locations show instantaneous and lasting effects on the smaller scales. There are instantaneous effects on larger scales, but they quickly dissipate. These effects hold across locations and more importantly across aggregation and non-aggregation sites.

The bottom row shows that when the largest scale is entered first (thus, the effect of smallest scale is conditioned on all other scales), the effect of the smallest scale is negligible on the middle and larger scales. However, there is a small effect on the small scales (i.e., negative values of qresp). For the non-aggregation locations (4–6), this effect is instantaneous and then goes to zero. For the aggregation locations (1–3), the effect is sustained across multiple time steps. Even when all other scales are in the model, the aggregation locations show an effect of the smallest scale on small scales. The non-aggregation locations do not show these effects.
Fig. 5. Impulse response functions: Smallest scale effects. The impulse response functions for effects of the smallest scale \( q = -4 \) on all scales. Each column of panels shows the effects for a single region. The upper row shows responses when the order of entry is smallest scale to largest scale (i.e., \( q = -4 \) to \( q = 3.5 \)). The lower row shows responses when the order of entry is largest scale to smallest scale (i.e., \( q = 3.5 \) to \( q = -4 \)). Within each panel, the \( z \) dimension shows change in \( D(\Delta) \), as a function of time step, and scale (i.e., qresp). Twenty steps of the model are shown.

Fig. 6. Impulse response functions: Middle scale effects. The impulse response functions for effects of the middle scale \( q = 0 \) on all scales. Each column of panels shows the effects for a single region. The upper row shows responses when the order of entry is smallest scale to largest scale (i.e., \( q = -4 \) to \( q = 3.5 \)). The lower row shows responses when the order of entry is largest scale to smallest scale (i.e., \( q = 3.5 \) to \( q = -4 \)). Within each panel, the \( z \) dimension shows change in \( D(\Delta) \), as a function of time step, and scale (i.e., qresp). Twenty steps of the model are shown.

Fig. 6 shows the impulse response functions for effects of the middle scale, \( q = 0 \). The top row of panels shows that when the smaller scales are entered first, there is a small positive effect on the larger scales. This pattern holds across all locations. There is also a negative effect on the smaller scales; only location 2 deviates from this pattern. The bottom row of panels shows that when the larger scales are entered first, the middle scale has no effect on the larger scales, but strong positive effects on the smaller scales. These different effects, depending on the ordering of the variables, have a straightforward interpretation. Given that the order of entry is smallest to largest (upper row), when the middle scale enters it is the largest scale on that step, and thus captures substantial effects of the larger scales. This suggests, that on average the larger scales appear to decrease the smaller scale dimensions \( D \). When the order of entry is largest to smallest (lower row), the middle scale enters as the smallest scale and therefore captures substantial effects of the smaller scales. On average, the effect of the smaller scales is to increase the small scale dimensions \( D \). There do not appear to be differences in the effects of the middle scale between the aggregation and non-aggregation locations.

Fig. 7 shows the impulse response functions for effects of the largest scale, \( q = 3.5 \). The upper row of panels shows the effects when this scale is entered last. When entered last, the effects of the largest scale on the middle and large scales is small or near zero. The effects on the smaller scales are more interesting. For aggregation locations, 1–3, the effect of the largest scale is an increase in the smaller scale dimensions \( D \). However, this effect does not obtain for the non-aggregation locations, 4–6. For aggregation locations, the largest scale has unique positive effects on the smaller scales. The bottom row of Fig. 7 shows that, when the larger scales are entered first, there are instantaneous effects at all scales (which is a
3.2.4. Forecast error variance decomposition

A different view of the effects of each scale on all other scales can be obtained through forecast error variance decomposition. This representation presents the effects of innovations from each scale as a proportion of the total variance in the affected scale, where the total variance in the affected scale is the sum of the squared effects. Because the impulse response functions for the three regions within each type (i.e., aggregation versus non-aggregation) were quite similar, we averaged the estimated variance decomposition for aggregation and non-aggregation regions.

Fig. 8 shows the forecast error variance decomposition when the order of entry is from smallest to largest. The nine panels in the top row show the average responses for the non-aggregation regions for each of the nine levels of $D(q)$. The nine panels in the bottom row show the average responses for the aggregation regions. Each panel shows the response of a different level of $D(q)$. The different colored stacked bars show the proportion of variance in that response accounted for by innovations from different levels of $D(q)$. A few things are evident in these two sets of panels. First, the smallest scale accounts for a larger proportion of variance in the smaller and middle scales. As will be clear in a moment, this is largely due to the order in which the variables are entered. However, despite the very conservative nature of this analysis with regards to the effect of the larger scales (the larger the scale, the later its entry), a substantial proportion of variance is accounted for by the larger scales. This is seen most dramatically in the responses of the larger scales themselves, the three columns of panels on the right. But even the smallest scales show effects of the larger scales.

Although there are obvious commonalities amongst the patterns of effects for the aggregation and non-aggregation regions, there are also considerable differences. The effects of the largest scale on the smallest scale are greater for the aggregation regions. Also, the middle scale effects ($q = -1, 0, 0.05$) are larger for the non-aggregation regions.

Fig. 9 shows the forecast error variance decomposition when the order of entry is from largest to smallest. Analogously to Fig. 8, each panel shows the response of one level of $D(q)$ to innovations in all levels. Because the largest scale effects are entered first, it is not surprising that they account for the majority of variance in the larger scales, and substantial variance in the middle and smaller scales. More interestingly, the smaller scales account for substantial variance in the smaller scale responses. The aggregation regions show the effects of the smaller scales on the small scales (left-hand panels) much more strongly than the non-aggregation regions.

In summary, the forecast error variance decomposition shows that innovations in the smaller scales have a greater effect on the small scales for the aggregation regions compared to the non-aggregation regions. Innovations in the middle scales generally have greater effects on the non-aggregation regions. These differences are seen clearly on the smaller scales for both analyses (positive first and negative first), but there are also differences for effects on the middle and larger scales when the order of entry is smallest to largest. Finally, innovations in the larger scales have greater effects on both the smaller scales for the aggregation regions than the non-aggregation regions.

3.3. Conclusions

We proposed that the multi-scale physical structures that constitute Dictyostelium discoideum are causally implicated in the formation of aggregates, a crucial event in their developmental cycle. To quantify these structures, we analyzed regions of images taken during an aggregation event using a multifractal formalism. For each region in each image in the sequence, this
analysis yields a spectrum of fractal dimensions that measures structures across multiple spatial scales. Fractal dimension is relevant because it relates to both the strength of the interactions among the structures and to the hydrodynamics of the media in which the structures reside. We showed that all six of the sampled regions had peaked $f(\alpha)$ functions and monotonically decreasing $D(q)$ functions across the 540 image sequence. This pattern, indicative of multifractals, held regardless of whether the regions ultimately contained an aggregation.

We selected nine $D(q)$ scales and treated them as a multivariate time series (over the 540 images), using vector autoregression/error-correction methods to model the across-scale interactions. Our results showed that for each region all nine scales contributed to maintaining the system’s equilibrium. Specifically, the fit of the model was significantly poorer when any of the scales was eliminated from the error-correction terms. Likewise, all the scales appear endogenous to the system. Removing the effect of the error-correction terms on any scale decreased the fit of the model. Aggregation and non-aggregation regions both showed these effects.

We used impulse response functions and forecast variance error decomposition to represent the effect of individual scales on the future states of all scales. There was strong agreement for the effects within type of region (i.e., aggregation vs. non-aggregation), as well as some commonalities across types. Perhaps most importantly, the aggregation and non-aggregation regions also showed systematic differences in the pattern of effects. Specifically, innovations in the smallest and largest scales had greater effects on the smaller scales for regions in which aggregation occurred. Innovations in the middle scales had greater effects on the non-aggregation regions.

Takentogether, these results show that structures at multiple scales self-regulate the system, keeping the relationships among the structures stable. At the same time, the effects of the individual-scale interactions are different for the aggregation and non-aggregation regions. Whether the differences in these interactions explain the differences in the macroscopic behavior of the system (i.e., aggregation versus non-aggregation) is not clear from the current study. However, there is a theoretical basis for interpreting the effects as causal. A substantial body of work on rheological dispersions has established
that the fractal dimension of clusters within the dispersions affect aggregation. Given this basis, the current results have a reasonable interpretation. For example, the finding that the largest scales have greater effects on the smaller scales for the aggregation regions is consistent with the idea that the developing aggregates change the dynamics of their constituent small-scale structures. The current work suggests that in Dictyostelium interactions amongst physical structures across a broad range of spatial scales govern macroscopic behavior.

Finally, we note that the approach we have taken here is motivated by the theoretical assertion that biological systems have nested physical architectures [34]. Under this view, structures at any particular scale of measurement are constitutive of larger scale structures, and constituted by smaller scale structures. Thus, unlike a mechanical system in which structures are independently defined [35], biological systems have a mutual dependence among their structures that is a consequence of their nested architecture. Given this architecture, it seems reasonable to suggest that fluctuations across these nested structures are mutually and reciprocally involved in regulating and altering the behavior of the system. The current manuscript represents an attempt to quantify the structures of Dictyostelium in a way that respects the nested nature of the architecture, and models the effects of changes in these quantities. This approach stands in contrast to standard approaches to biological systems in which structures are treated like parts in a mechanical system, and effects are modeled as in a causal chain. We suggest that developing an approach capable of capturing the interactions in nested architectures, such as those in biology, will be an important step in investigating such systems.

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References


