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Title: Measuring Collective Behaviour of Multi-Cellular Ensembles: Role of Space-Time Scales

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Keywords: Collective behaviour; Synchronisation; Multi-scale Modelling; Spatio-temporal dynamics

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Abstract: Living systems are spectacular examples of spatio-temporally organized structures. During development of complex organisation there is dynamic equilibrium between the local and global processes acting at intra- and inter-cellular levels in multiple space and time scales. Even though in modelling studies such spatio-temporal systems can be described at many different space-time scales and organizational levels, the experimental quantities measured and predictions useful for practical applications are at a macroscopic (coarser or averaged) level/scale that are limited by the resolution of measuring method and experimental protocol. In this work we address the question if the spatio-temporal collective dynamics exhibited by a multi-scale system can discriminate, or, be borne out from the coarse-grained and averaged measurements done at different spatial and temporal scales. Using a simple model of a ring of cells, we show that measurements of both spatial and spatio-temporal average behaviour in this multi-cellular ensemble can mask the variety of collective dynamics observed at other space-time scales, and exhibit completely different behaviours. Such outcomes of measurements can lead to incomplete and incorrect understanding of physiological functions and pathogenesis in multi-cell ensembles.

Response to Reviewers: See attachment.

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9 **Measuring Collective Behaviour of Multi-Cellular Ensembles:**
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ABSTRACT

Living systems are spectacular examples of spatio-temporally organized structures. During development of complex organisation there is dynamic equilibrium between the local and global processes acting at intra- and inter-cellular levels in multiple space and time scales. Even though in modelling studies such spatio-temporal systems can be described at many different space-time scales and organizational levels, the experimental quantities measured and predictions useful for practical applications are at a macroscopic (coarser or averaged) level/scale that are limited by the resolution of measuring method and experimental protocol. In this work we address the question if the spatio-temporal collective dynamics exhibited by a multi-scale system can discriminate, or, be borne out from the coarse-grained and averaged measurements done at different spatial and temporal scales. Using a simple model of a ring of cells, we show that measurements of both spatial and spatio-temporal average behaviour in this multi-cellular ensemble can mask the variety of collective dynamics observed at other space-time scales, and exhibit completely different behaviours. Such outcomes of measurements can lead to incomplete and incorrect understanding of physiological functions and pathogenesis in multi-cell ensembles.

KEY WORDS:

Collective behaviour;

Synchronisation;

Multi-scale Modelling;

Spatio-temporal dynamics.

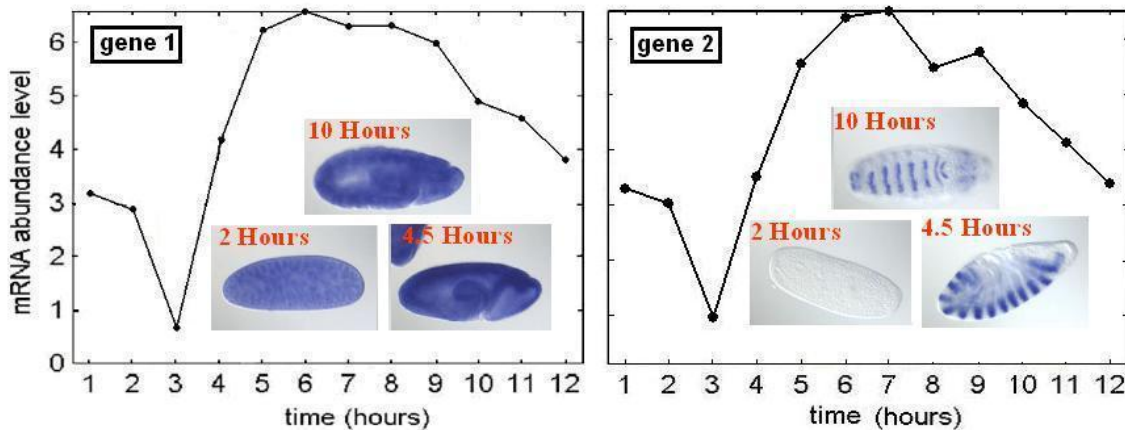
1. INTRODUCTION

Multi-cellular ensembles constitute groups of many cells. In a population, individual behaviour of the cells may be uncorrelated and the population behaviour is a simple sum of the individual behaviours. When cells in a population interact, the individual behaviour of the cells is regulated by the interaction, and the ensemble can show a unified collective behaviour, which is not just a "sum of the parts". Interactions can occur through the response of the individual cells in a population to concentration of external molecules in their environment. Examples are - Community Effect, Quorum Sensing, and Biofilms, where a population of similar cells exhibits cell density-dependent induction of new gene expression in response to an external signal factor (Freeman and Gurdon, 2002; Fuqua, *et al*, 1996; Costerton, 2004; Miller and Bassler, 2001; Garcia-Ojalvo, *et al*, 2004). Cells in tissues and organs are examples of structured ensemble of cells, where they interact directly through membrane-bound molecules, gap junctions, diffusion of secreted chemicals, etc (Suguna and Sinha, 2005). The cardiac and muscle tissue, islets of Langerhans in pancreas, plant roots, etc, are just a few examples of the varied types of structures that are formed in this type of multi-cellular ensembles (Murray, 1989). Here, the arrangement of cells and the types of contacts complement their specific functions. Collective dynamics of the structured multi-cellular ensembles may have both space and time components giving rise to spatio-temporal dynamics and patterns.

Multi-cellular ensembles are "multi-scale systems", i.e., they contain components that span multiple length and time scales. At the individual cell level, complex intra-cellular network of interacting biochemical pathways govern the functional behaviour of the cells. Inter-cellular communication is a necessary prerequisite for effective collective functioning in the multi-cellular ensembles. Emergence of the observed macroscopic (collective) behaviour arises from the type and strength of the interactions among the constituent cells and their local cellular behaviour. Thus the average behaviour of the cellular ensemble is a result of both intra- and inter-cellular processes that happen at different space and time scales (Alarcon, *et al*, 2005). Biological function can also be studied at multiple levels – i.e., at different levels of abstraction (molecular, genetic, cellular, organismal, social/ecological). For example, the collective behaviour of chemotaxis in bacterial populations provides an example of how cell-

level decision-making (signal detection, transduction, and swimming behaviour) translates into population-level behaviour spanning time scales from 10^{-2} second to days (Erban and Othmer, 2005). The role of intrinsic and extrinsic stochasticity and interaction with the environment at different organisational levels also has significant effects in the function of these ensembles (Sinha, 2005; Maithreye and Sinha, 2007). Given the multi-scale and multilevel nature of biological processes, when a study is focused to a specific level/scale based on the question to be addressed, an important question arises if the net effect of the smaller-faster scales on the larger-slower scales can be measured when studied at the specific larger-slower scales. Or, in other words, *how much information can get truncated or unresolved at some scale resulting in the loss of predictability at the other scales.*

Important information can also be lost when, in a spatio-temporal process, measurements are made only at any one dimension - either spatial or temporal. An example (Myers, personal communication; Peng, *et al*, 2007) is shown in Fig.1, where the expression of two genes (gene 1 and gene 2) have been studied by measuring their mRNA abundance levels for 12 hours in whole *Drosophila* embryos. The temporal expression profiles of gene 1 and gene 2 (line plots in Fig.1) indicate that the average expression levels of the two genes in whole embryos during specific developmental phases are highly correlated (correlation coefficient is 0.98). The insets in both the plots show the spatial pattern of expression of the



Courtesy Gene Myers & Hanchuan Peng, HHMI Janelia Farm

Fig.1: Measurement of the expression of two genes showing average temporal expression profile (line plot) and their spatio-temporal expression (inset).

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6 two genes in the embryos at three different times, and it is very clear that their spatial
7 expression pattern over time is totally uncorrelated. This example shows how, during
8 experimentation, absence/truncation (spatial averaging) of information at the spatial scale can
9 lead to a loss of information at the temporal scale leading to incomplete or even incorrect
10 inferences with regard to co-expressions of the two genes during development.

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14 Modelling multilevel/multi-scale processes pose difficult mathematical and
15 computational problems, and are an active area of research now. To incorporate individual-
16 level behaviour into population-level models, through the combination of microscopic
17 description with macroscopic changes on a coarse grid, involves the use of different sorts of
18 mathematics for their descriptions: continuous time Markov processes, discrete mechanics,
19 continuum mechanics, discrete time processes, etc (Shenoy, *et al*, 1999; Garcia, *et al*, 1999;
20 Kevrekidis, *et al*, 2003; Setayeshagar, *et al*, 2005). In modelling of biological processes,
21 traditionally first the macroscopic evolution equations for each variable of importance is
22 derived from the microscopic processes based on the existing experimental information.

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Different mathematical and computational tools are then used to derive the macroscopic descriptions. The implication is that the macroscopic rules, a description of the system at a coarse-grained, higher level of organisation, can be deduced from microscopic rules (Kevrekidis, *et al*, 2005). But, in general, the questions of interest that require practical answers are asked at the macroscopic ("system") scale, whereas, the multi-component model description is at a much finer scale, which can be explicitly tracked in a simulation. Sometimes it is also unclear at which level of observation can one be practically predictive. Thus, while extending the modelling and simulation results to experimental observations, it is necessary to be aware of this gap between the two. The inferences drawn would be only as good as the data on which they are based, which, in turn, depends on the important problem of "Resolution" and "Granularity" of experimental measurements, which reflect the scale at which the processes are measured.

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The two most important features of collective dynamics in any multi-component, coupled system are - synchrony and spatio-temporal patterns (Pikovsky, *et al*, 2001; Murray, 1989). Here the dynamics exhibited by the 'whole' ensemble can be quite different from the individual components' behaviour (Bertram, *et al*, 2000). Also spatial patterns can set up in the

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5 structured ensembles (*e.g.* embryos, cardiac tissue) whose length scale spans that of its several
6 components (Bub, *et al*, 2005). Usually while making experimental measurements of any
7 observable quantity in a cell population or tissue a sum total of the concentration is measured
8 from the whole ensemble, as it is difficult to observe the individual variation of end-product
9 concentrations in each cell. The underlying assumption is that the total concentration
10 measured (output 'signal') will give representative information of the dynamics of the cellular
11 ensemble. The precision of experimental measurements is limited by many factors, such as the
12 response times and the resolution limit of the instruments used, and also on the sampling rate
13 of the measurements. If the instrument has a low time-resolution, we would get the time
14 average of the 'signal' concentration around the sampling instant. Also, experimental
15 measurements are not instantaneous and are made over a range of time. In such cases one can
16 only measure the spatiotemporal average of the end product. Thus, experimental
17 measurements in the multi-cellular ensemble often comprise of spatial averages of the variable
18 of interest, which may also be time-averaged around the sampling time. Hence, it is not clear if
19 the different types of spatio-temporal collective dynamics exhibited by a multi-scale system
20 can be discriminated, or, be borne out from the coarse-grained and averaged measurements
21 done at different spatial and temporal scales.
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36 The focus of this paper is the study of the role of scales of measurement in the faithful
37 description of collective spatio-temporal patterns and processes in a multi-cellular ensemble.
38 To study this we have taken the following simple theoretical approach. First, we consider a
39 ring of cells as a model for a simple multi-cellular ensemble, where each cell interacts with two
40 nearest neighbouring cells on the ring through inter-cellular diffusion of the end product of an
41 intra-cellular biochemical pathway. Our earlier work on this pathway showed that the
42 dynamics of the end product in each cell is determined by the kinetic and stoichiometric
43 parameters of the intra-cellular pathway (Sinha and Ramaswamy, 1987, Suguna, *et al*, 1999).
44 The spatio-temporal collective behaviour of the end product in this model multi-cellular
45 ensemble (*i.e.*, the ring of cells) was studied for different diffusive coupling strengths and ring
46 size (*i.e.*, the number of cells). It was shown that this ring of cells exhibits different types of
47 global collective dynamics and/or spatial patterns of the end product concentration under
48 different conditions when studied at the lowest time and space scale, which is not always the
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same as the individual cell's dynamics (Suguna and Sinha, 2005; Rajesh, *et al*, 2007). To address the role of resolving power of measurements in discriminating between the distinctly different collective dynamics of the ring, we now study the role of spatial and temporal averaging of the variable of interest (end product of the pathway, here) at these different behavioural regime. We show that the spatio-temporal averaging of these 'signals' in different synchronisation states can have non-intuitive temporal features, and can either mask the differences, or show a totally different behaviour leading to inconsistent inferences. This has important bearing in the understanding and prediction of collective functional behaviour in cellular ensembles from experimental data vis-à-vis theoretical analysis.

2. MODELS AND METHOD

2.1 Single Cell Model

Each cell in the model multi-cellular system, i.e., the ring of cells, consists of a three-step activator-inhibitor biochemical pathway, where, the substrate S1 is converted to S3 through an intermediate substrate S2 (see Fig.2a). The flux through the pathway is regulated by two feedback loops - the end-product inhibition of S1 by S3 (negative feedback), and the auto-catalytic production (positive feedback) of S3 from S2 mediated by an allosteric enzyme, E. The details of the model have been studied earlier (Sinha and Ramaswamy, 1987; Suguna, *et al*, 1999).

The time evolution of the three substrates S1, S2, and S3 of the pathway can be described by the following dimensionless equations:

$$\begin{aligned}\frac{dx}{dt} &= F(z) - kx; \\ \frac{dy}{dt} &= x - G(y, z); \\ \frac{dz}{dt} &= G(y, z) - qz,\end{aligned}\tag{1}$$

where, x, y, and z are the normalized concentrations of the substrates S1, S2 and S3. The first order, non-saturated rates of the degradation reaction of S1 and S3 are k and q. The non-linear functions F(z) and G(y,z) represent the negative and positive feedback processes and are

found to be as follows:

$$F(z) = 1/(1+z^n), \quad G(y,z) = Ty(1+y)(1+z)^2/[L+(1+y)^2(1+z)^2],$$

where, L and T are the allosteric constant and maximum velocity of the enzyme, E, respectively.

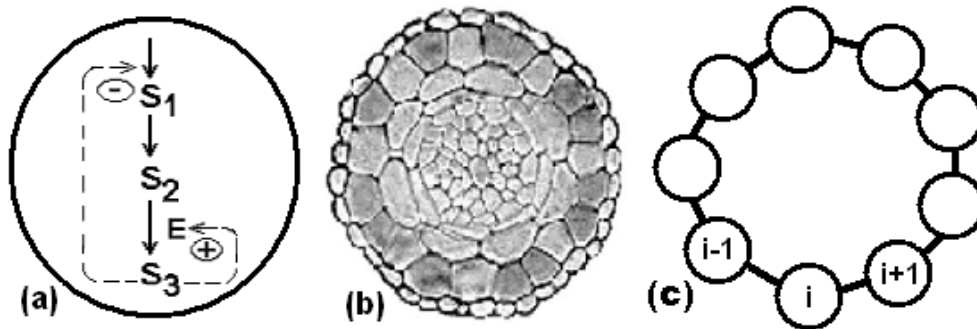


Fig.2: The model multi-cellular ensemble – (a) A single cell incorporating a three-step biochemical pathway; (b) Circular arrangement of cells in plant root section; (c) A model ring of cells where each cell (node i , as shown in (a)) is diffusively coupled to its nearest neighbouring cells (nodes, $i+1$ and $i-1$).

The parameter values used in this model pathway are chosen based on pathways incorporating positive and negative feedback processes such as, the cell cycle, glycolytic cycle, and cAMP oscillations in slime molds (Goldbeter and Nicolis, 1976; Tyson, 1983). The basal parameter values are: $n=4$, $L=10^6$, $T=10$, $k=1$, and $q=0.01$, where it shows a simple limit cycle oscillation. The dynamics exhibited by this pathway is a function of the parameters (Suguna, *et al*, 1999), and the parameters chosen for this study are $q=0.1$, and $k=0.003$, where the end product dynamics is chaotic (Sinha and Ramaswamy, 1987; Suguna and Sinha, 2005).

2.2 Model of the Multi-Cellular Ensemble: Ring of cells

For the multi-cellular ensemble we consider a simple model where the cells are assumed to form a closed ring-like lattice structure, as is observed in cells in a plant-root (Fig.2b). Each cell in this one-dimensional lattice of N cells (see Fig.2c), has the model biochemical pathway described by Eq.(1) (shown in Fig.2a), and interacts through the diffusion of a fraction of the end product, z , from each cell (i) to its two neighbouring cells ($i+1$ and $i-1$).

The time evolution of the pathway in this ring of N cells (with periodic boundary conditions) is given by the following equations:

$$\begin{aligned}\frac{dx^i}{dt} &= F(z^i) - kx^i; \\ \frac{dy^i}{dt} &= x^i - G(y^i, z^i) \\ \frac{dz^i}{dt} &= G(y^i, z^i) - qz^i - \varepsilon(z^i) + (\varepsilon/2)*(z^{i-1} + z^{i+1})\end{aligned}\tag{2}$$

where, ε ($0 < \varepsilon < 1$) is the diffusive coupling strength of the end-product to its nearest neighbours. The N cells are indexed as $i=1, \dots, N$.

The spatio-temporal behaviour of this multi-cell system is simulated using a discretization scheme in the coupled map lattice approach (Oono and Puri, 1987; Kaneko, 1993). Simulation is done using the fourth order Runge-Kutta scheme (Press, *et al*, 1992), and data visualisation using MATLAB (<http://www.mathworks.com>). All studies have been carried out with a minimum of fifty different random initial conditions uniformly distributed around the unstable steady state ($z^* \pm 2$, $z^* = 5.163$). Simulations have been performed for $t=10^5$, and results are generally presented for the end product, z, for the last 5000 time units.

3. RESULTS

In the following sections, we first give a concise description of the pathway behaviour in single cells on the ring when uncoupled. On coupling through diffusion, the collective behaviour of the cells in the ring depends on the size of the ensemble (N) and the rate of diffusion (ε). We present the spatio-temporal behaviour exhibited by the ring of coupled cells with varying N and ε (for details, see Rajesh, *et al*, 2007). We then show how different time scales of measurements affect the resolution of the underlying ensemble behaviour at these behavioural regimes.

It may be mentioned here that this pathway exhibits a wide range of dynamics—equilibrium, limit cycle, period-doubling, birhythmic, complex, and chaotic oscillations—with variation in parameters, and the complex and chaotic dynamics is observed in larger parameter space as the co-operativity of negative feedback (n) increases (Suguna, *et al*, 1999). Earlier study (Suguna and Sinha, 2005) with coupled cells at different dynamics regimes showed that

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6 – (i) the global dynamics in the coupled cells are fully synchronised, with the same periodicity
7 and amplitude as the individual oscillatory cells. Thus, the collective dynamics of the coupled
8 cell system is identical to that of a single cell in this regime; and (ii) cells at birhythmic states
9 synchronized to the faster oscillation type for larger lattice size.
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14 **3.1 Dynamics of Uncoupled Cells**

15 For the kinetic parameters chosen, the pathway in each cell exhibits highly irregular,
16 fluctuating, chaotic dynamics in the substrate and end product concentrations (Suguna, *et al*,
17 1999). In Fig.3 the local and ensemble dynamics of 50 uncoupled cells are shown. Fig.3a
18 shows the (y-z) phase portrait of this irregular oscillations, and Fig.3b the broadband nature of
19 the log-power spectrum, of the 25th cell on the ring, which is indicative of the diverse time-
20 scales of oscillations associated with the chaotic dynamics. Fig.3c shows the superposition of
21 the time series of the end product, z, for all 50 cells. As a result of their sensitivity to initial
22 conditions, a generic property of chaotic oscillations, the time series of z in these cells are
23 totally unsynchronized and uncorrelated. Fig.3d shows the space-time image plot of the
24 uncoupled ensemble, where the X-axis plots the number of cells, and the Y-axis shows the
25 time evolution of z in each cell. The colour bar denotes the value of z. It is clear here that, like
26 Fig.3c, cells evolve independently with their intrinsic chaotic dynamics.
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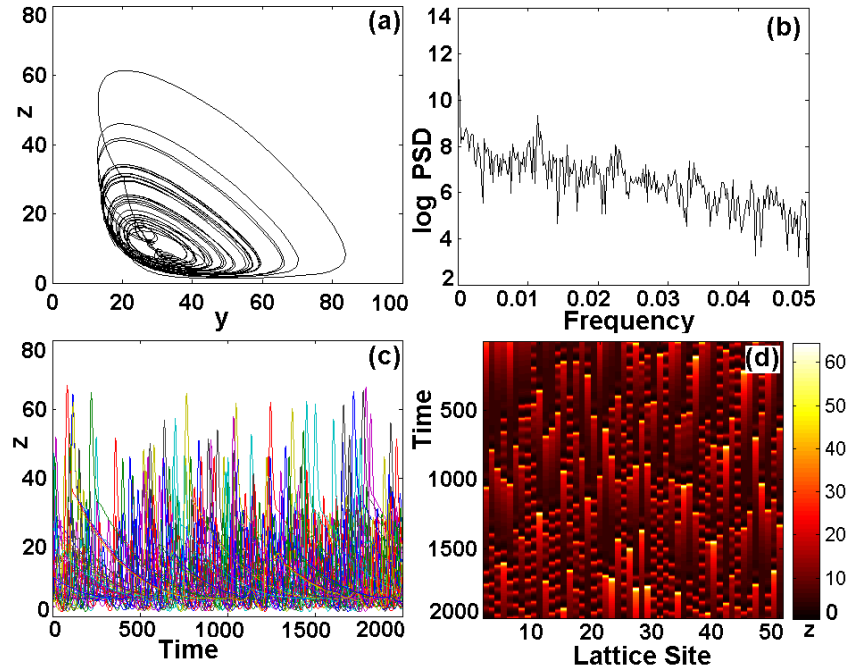


Fig. 3. Dynamics of 50 uncoupled cells. (a) $(y-z)$ Phase portrait, and (b) Power spectrum of the 25th cell. (c) Superposition of time series, and (d) Space-Time plot, of all the cells.

3.2 Spatio-temporal Behaviour of the Ring of Cells

When these cells exhibiting chaotic dynamics interact to their nearest neighbours on the ring through diffusion of the end product, z , their individual and collective behaviour depends on the size of the ensemble (N) and the rate of diffusion (ϵ). We first show the role of system size (number of cells, N) in the emergent collective behaviour, and then show the spatiotemporal dynamics of the cells for different diffusive strength (ϵ) for a fixed number of cells in the ring.

3.2a Effect of the number cells in the ring (N)

To study the different types of collective dynamics exhibited by the ring of cells of different sizes, simulations have been done on lattices of size $N = 10$ to 100 in multiples of 10. Two features are noted while studying how the inclusion of more cells affects the dynamical behaviour of the cells in the ring – the dynamics exhibited by the individual cells in the ring, and the global spatiotemporal behaviour of the ring of cells.

Examples of the different types of collective dynamics exhibited by the ring of cells of different sizes (N) are shown in Fig.4. The left column shows the superposition of time series of z in all the cells. The middle column shows the space-time plot of z with concentration being colour-coded (colour bar scale shown). To characterize the change in the local dynamics of the cells, the right column shows the major frequency components of the dynamics in one of the cells (cell number= $N/2$) in its power spectrum.

Four types of global dynamics are observed for different N in the ring of cells–

- (i) *Complete Synchronisation* (CS): For small ring sizes (e.g., $N=10, 20, 30$), all cells in the ring are completely synchronised in their behaviour - both in phase and amplitude - and they all exhibit chaotic dynamics. Fig.4a shows the superposition of time series plots of 10 cells (for $\varepsilon = 0.72$), which gives a single trajectory implying that concentration of z in all the cells are changing in exact synchrony. The space-time plot (Fig.4b) also confirms the complete synchronization as z concentrations in all cells on the ring are perfectly in line. The power spectrum (Fig.4c) of the 5th cell's time series confirms the chaotic nature of the dynamics through its broadband nature. This indicates that the local and global dynamics are the same in small rings of cells, and the ring of cells acts like a coherent ensemble.
- (ii) *Intermittent Phase Synchronisation* (IPS): For larger size rings, the dynamics of all the cells are not perfectly correlated all the time. The phase synchrony is lost for certain intervals and it is regained within a short time. The individual behaviour of the cells remains chaotic. Fig.4(d-f) illustrate the intermittent behaviour of the ring for $N=70$ and $\varepsilon = 0.72$. Both the time series plot (Fig.4d) and the space-time plot (Fig.4e) show that the cells' dynamics are not perfectly correlated all the time. Here, the cells switch between synchronised and unsynchronised states (intermittency) in irregular intervals. The individual cell's dynamics is chaotic as seen from the broadband nature of the power spectrum (Fig.4f).

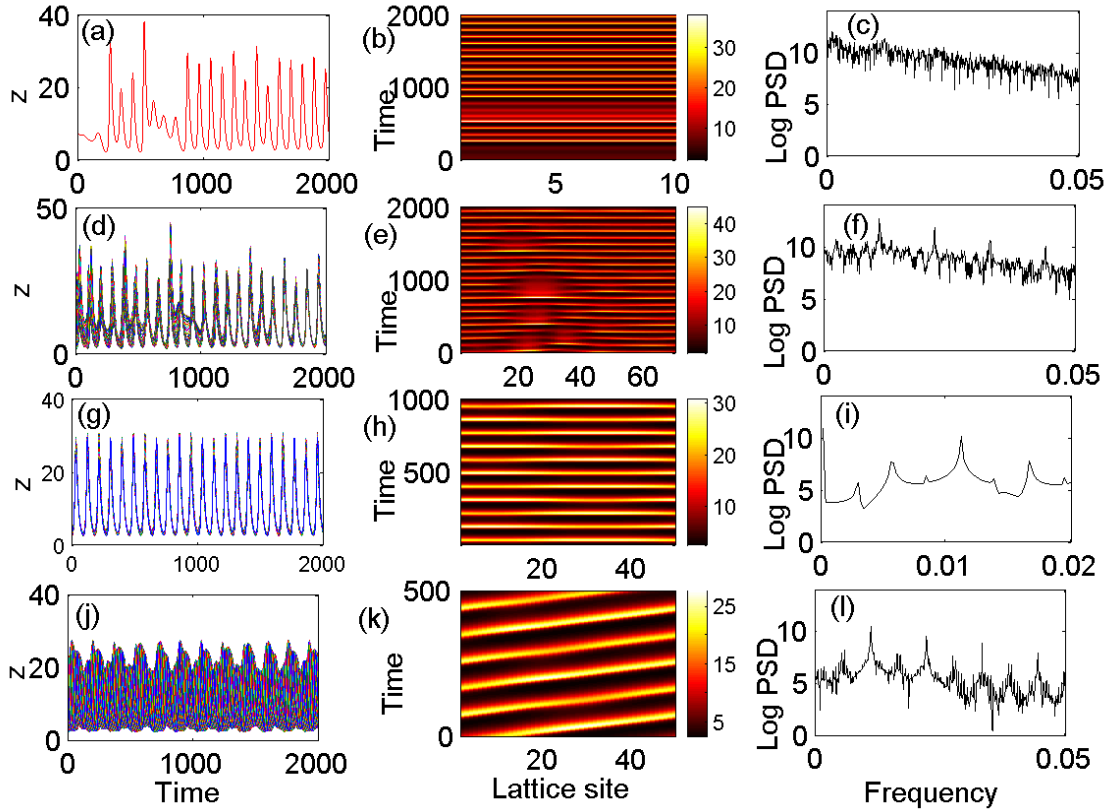


Fig.4: Collective behaviour of coupled cells. Left column: superposition of time series of all cells; Middle column: space-time image of the ring of cells; Right column: Power Spectrum of the time series of z of the $(N/2)^{\text{th}}$ cell in the ring. $\varepsilon=0.72$ for (a)-(i), and $\varepsilon=0.6$ for (j-l).

(a-c) Complete synchronization, $N=10$. (d-f) Intermittent phase synchronization, $N=70$.

(g-i) Periodic phase synchronization, $N=50$. (j-l) Travelling wave, $N=50$.

(iii) *Phase Synchronisation (PS)*: Though IPS is the most common form of global collective dynamics exhibited by larger size rings, occasional cases of other types of dynamics, such as *Phase synchronisation* and *Travelling waves* are also observed for larger ensembles. In Fig.4(g-i), for $N=50$, $\varepsilon=0.72$, the ring of cells exhibits an interesting case of *phase synchronisation with phase slip*, where, even though the chaotic dynamics in each cell is completely suppressed, and they show stable periodic (Period 4) oscillations, but all the cells in the ring do not oscillate at the same phase, but are phase-locked to be in a synchronised state. There is a phase entrainment with a spatial pattern of phase slips that

allows the global dynamics of the ring of cells to be “two high peaks followed by two lower peaks” (see Rajesh, *et al*, 2007 for details). The superposition of time series plots of all 50 cells is shown in Fig.4g. The space-time plot (Fig.4h) and the power spectrum (Fig.4i) confirm the phase synchrony and periodic nature of the oscillations. The broadband nature is absent in the power spectrum since chaos has been suppressed and the individual cells show a higher periodic oscillation.

(iv) *Travelling Waves* (TW): Fig.4(j-l) shows an example of the other type of collective dynamics -*travelling waves* of the end product concentration through the ring of cells, for $N=50$ and $\epsilon=0.6$. Here the concentration of z (phases of the oscillation) in the cells is distributed throughout the ring as a spatially non-localized structure, such that the spatio-temporal evolution of it gives rise to a *travelling wave*. The peak of the wave travels through the circular lattice with almost uniform velocity (Fig.4k), while the individual cell dynamics remains chaotic (Fig.4l).

3.2b Effect of Coupling Strength, ϵ

The strength of inter-cellular interaction also plays a role in determining the collective behaviour of the ring of cells. We studied the spatio-temporal dynamics of a ring of cells ($N=50$) for the range of coupling strength $0 < \epsilon < 1$ at a step of 0.1. To include the effects of the intrinsic variability in the intracellular concentrations in different cells, we studied the spatio-temporal dynamics of the ring of cells for fifty different initial values of the pathway metabolites for every ϵ . Fig.5 shows the collective behaviour exhibited by the ring of cells for different coupling strength (ϵ in Y axis) for the 50 initial conditions (in X axis). It is clear from the figure that Intermittent Phase Synchronisation (IPS in grey) is the predominant collective behaviour at low and medium strength of diffusive coupling for the ring of 50 cells. But the ring does show phase synchronisation (PS in white) with individual cells having periodic dynamics at higher coupling strengths ($\epsilon=0.7, 0.8$). In few cases the existence of travelling waves (TW in black) are also observed. Since complete synchronisation (CS) is seen only in rings of small number of cells, no such dynamics is observed here.

In all the above cases, the dynamics of z in the individual cells, when coupled in the ring, mostly remains chaotic, and some times changes to periodic (period 4 for PS). But the

ring of cells, as an ensemble, shows a variety of collective behaviour as is seen in Fig.4. Thus the effect of inter-cellular diffusion can lead to setting up of different types of global spatio-temporal dynamical patterns spanning different space and time scales in multi-cellular ensembles of different sizes. Such interaction in an ensemble can also induce changes in the local dynamics of the individual cells from chaotic to periodic, thereby suppressing the intrinsic behaviour of the cells.

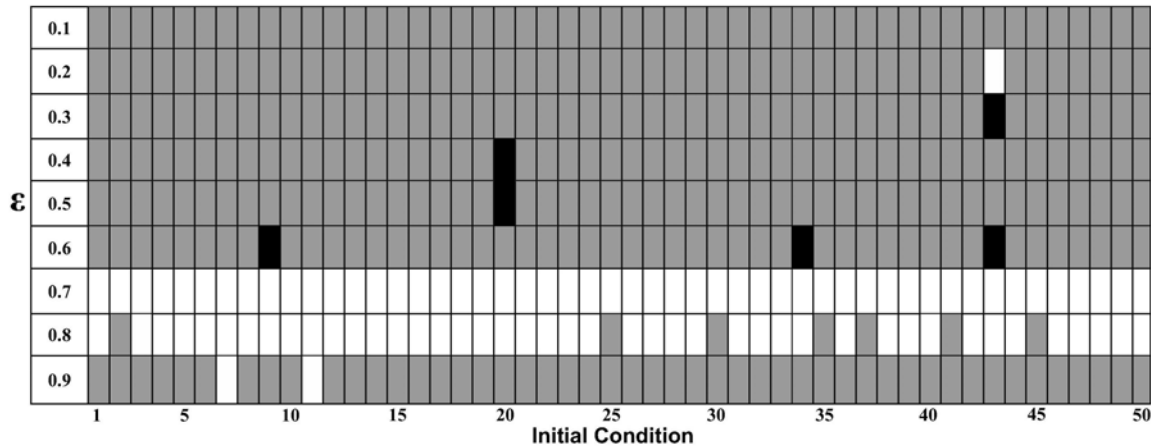


Fig.5. Variety of collective dynamics exhibited by the ring of cells ($N=50$) for different ϵ . Colour code for dynamics - grey: IPS; white: PS; black: TW. See text for details.

3.3 Measuring Collective Behaviour

Through simulation we can track the end product (z) concentration in the individual cells and also for the ring of cells, at different dynamical states. Here we address the role of the resolving power of experimental measurements in discriminating between these distinctly different collective dynamics of the ring. Generally, experimental measurements of z in the ring of cells would involve sampling of the total amount of z ('signal' or 'output') from the cells at scales that are limited by the instrumental precision and measurement protocols. Thus, experimental measurements on the ring would comprise of spatial averages of z , which may also be time-averaged around the sampling time. In the following sections we have studied these two experimentally relevant features - spatial averaging and spatio-temporal averaging of measurements of the concentrations of z in the ring of cells - at different collective dynamical regimes as shown in Fig.4 (CS, IPS, PS, TW) along with the uncoupled cells (Fig.3).

3.3a Measuring the total output ‘signal’

The time series of the spatial average of z , Z_{av} , is plotted in Fig.6 for the uncoupled and coupled cells in the ring at different behavioural regimes. This would represent the total ‘output signal’ of z from the multi-cellular ensemble at different spatio-temporal dynamical states. The measurements have been done at every time step of simulations here.

Fig.6a shows that even though each cell in the uncoupled cell population shows large and irregular concentration changes of z , the ensemble output of z is relatively steady. This is because the concentration variation of z in the cells is phase-incoherent and asynchronous.

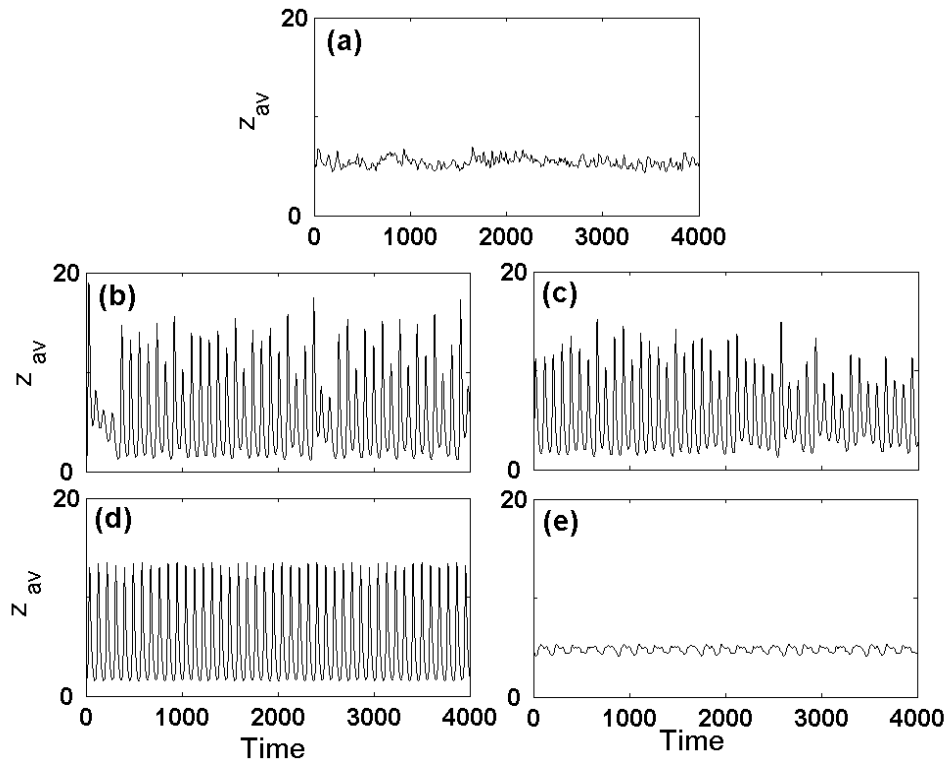


Fig.6: Time evolution of total output (Z_{av}). (a) Uncoupled chaotic cells;
 (b) Complete synchronised state; (c) Intermittent phase synchronised state;
 (d) Periodic synchronised state; (e) Travelling wave.

Thus, the underlying chaotic dynamics in the individual cells are not measurable from the total output signal, which shows small fluctuations around the mean value. The ‘output’ of z shown in Fig.6b and c, on the other hand, is almost indistinguishable, even though the underlying

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6 spatio-temporal dynamics of the ring of coupled cells are completely synchronised (CS) or
7 partially synchronised (IPS). Fig.6d shows that the average output from the ring when the cell
8 dynamics are phase synchronised (PS) is not different from the spatio-temporal behaviour
9 seen in Fig.4g. In the TW state, when there is a chaotic travelling wave front moving through
10 the ring, Z_{av} shows a totally different temporal behaviour (Fig.6e). The high amplitude
11 variation in z (Fig.4i) is completely suppressed, and a very small amplitude oscillation around
12 10 units is obtained in this case. The temporal behaviour of the Z_{av} in the uncoupled cells
13 (Fig.6a) and the coupled cells undergoing travelling wave dynamics (Fig.6e), show similar
14 trend. Thus from Fig.6, it is clear that the total ‘output signal’ of the multi-cell ensemble may
15 not be representative of either the single cell behaviour, or the collective spatiotemporal
16 dynamics, and the spatiotemporal pattern and dynamics can not be easily distinguished from
17 measuring the spatial average of the ensemble of cells.

28 **3.3b Role of time scales in the measurement of the spatial average of z**

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30 The precision of experimental measurements is limited by the response times of the
31 instruments and by the sampling rate of the experimenter. At low temporal resolution, one
32 gets the time average of the Z_{av} in the ensemble around the sampling instant. In such cases one
33 can only measure the spatio-temporal average of the end product for a time range. In general,
34 as expected, the fluctuations tend to smoothen out as the time scale of averaging is increased,
35 since the small-scale variations in the time series are lost. In Fig.7 to 9, we show the time series
36 of the output signal (Z_{av}) after averaging over different time intervals (50, 100 and 500 time
37 units), for the five collective dynamical states - uncoupled, CS, IPS, PS, and TW.

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39 For short sampling time (50 units in Fig.7), the temporal variations of Z_{av} are almost
40 indistinguishable for CS, IPS, and PS (Fig.7b,c,d), except the feature that the ring of cells
41 showing CS can sometimes show a large amplitude pulse of the output signal due to their
42 synchronised chaotic dynamics. Z_{av} in both uncoupled cells and coupled cells in TW exhibit a
43 rather regular output of same value with small fluctuations around the mean.

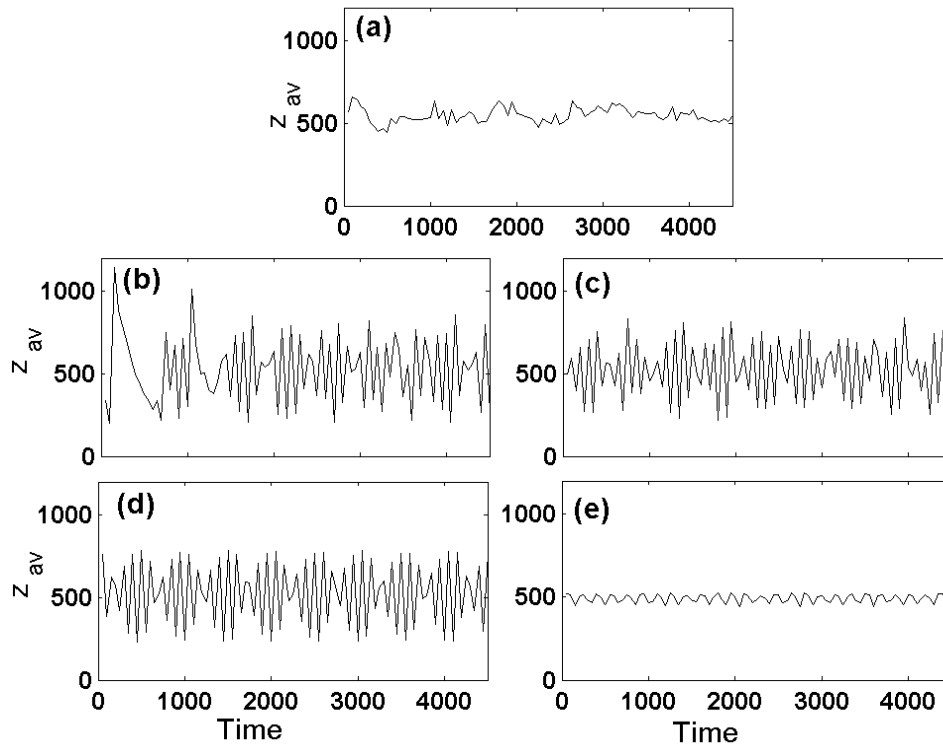


Fig. 7: Time evolution of output (Z_{av}) averaged over 50 time units: (a) Uncoupled chaotic cells; (b) Complete synchronised state; (c) Intermittent phase synchronised state; (d) Periodic synchronised state; (e) Travelling wave.

At larger sampling times (100 in Fig.8, and 500 in Fig.9), the temporal dynamics of the ‘output signal’ from the ring at CS, IPS and PS continue to show similar behaviour and tends to be similar to the uncoupled cells, except that the signals from the PS show more regular oscillations and CS exhibit occasional bursts. The ring showing TW dynamics gives a fairly constant regular output of Z_{av} , even though it is shown earlier that the individual cells show chaotic oscillations and the collective behaviour has concentration waves travelling along the ring.

Thus, our results show that the four different spatio-temporal dynamical states cannot be easily distinguished from the spatially averaged and spatio-temporally averaged measurements of the ‘output signal’. This is clearly seen when the ring of cells shows the non-stationary structure of Travelling Waves (compare Fig.4i, to Fig.6e, 7e, 8e, and 9e), where the large oscillation in the local temporal and global spatio-temporal dynamics are completely

suppressed in the population average measurements, giving only near-constant regular output. The multi-cellular rings with completely synchronised (Fig.7b, 8b and 9b) and intermittently synchronised (Fig.7c, 8c and 9c) dynamics tend to show indistinguishable temporal behaviour of the total ‘signal’, except for the existence of sudden highs in the former. In fact, for medium and high temporal averaging (100 and 500), the three different dynamical states of the lattice - CS, IPS, and PS - cannot be easily distinguished from the uncoupled cells in their total spatio-temporal output. The phase-synchronised-periodic-ring (PS) shows interesting transitions depending on the time range of sampling. It shows burst-like behaviour for both low and high temporal averages (Fig.7d and 9d), but exhibits a fairly regular output for medium temporal averaging (Fig.8d). Thus, the temporal behaviour of the total output ‘signal’ from the multi-cell ensemble may not faithfully represent its underlying spatio-temporal collective dynamics, and can exhibit different behaviour depending on the time of sampling.

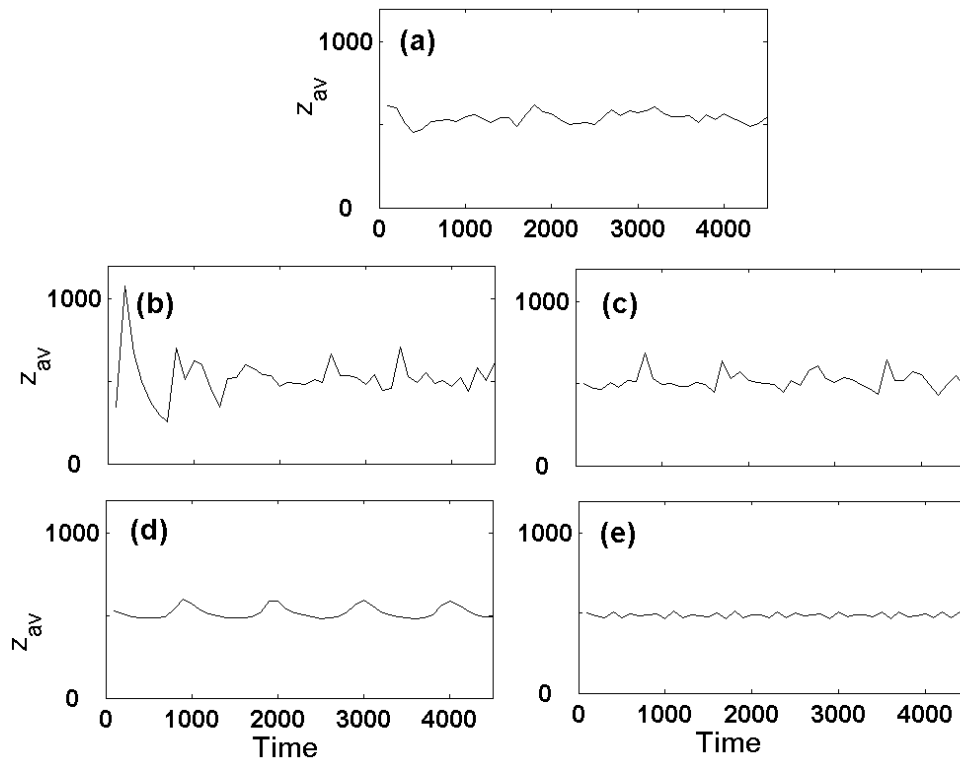


Fig. 8: Time evolution of output (Z_{av}) averaged over 100 time units: (a) Uncoupled chaotic cells; (b) Complete synchronised state; (c) Intermittent phase synchronised state; (d) Periodic synchronised state; (e) Travelling wave.

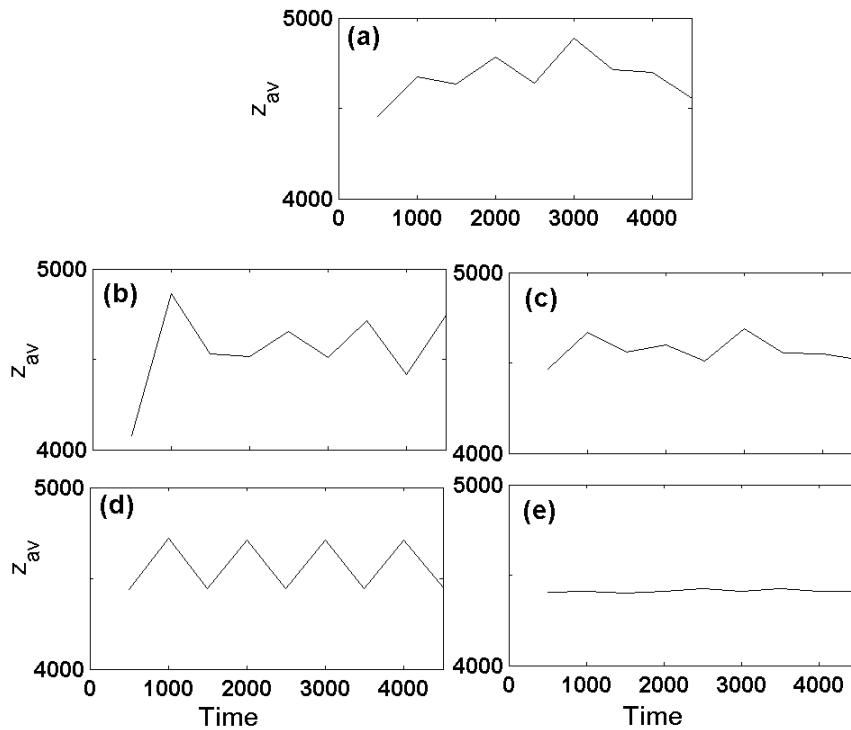


Fig. 9: Time evolution of output (Z_{av}) averaged over 500 time units: (a) Uncoupled chaotic cells; (b) Complete synchronised state; (c) Intermittent phase synchronised state; (d) Periodic synchronised state; (e) Travelling wave.

4. DISCUSSION

It is increasingly becoming apparent that many biological mechanisms act at various spatio-temporal scales in multi-cellular organisms, which requires multi-scale and multi-level measurements and modelling methods (Alber, *et al*, 2005; Chaturvedi, *et al*, 2005). Most studies (experimental, modelling and simulation) have focused on single levels or scales, e.g., genomic/proteomic, cellular, tissue, organ, whole body, etc. But each of these contains processes/components that span multiple length and time scales - from nanometers to meters and from nanoseconds to days, months and longer. The number of components can also be large (thousands of interacting molecules and ions) with nonlinear and stochastic interactions among them. Spatio-temporal patterns and collective dynamics are important features of complex multi-cellular processes/systems in normal and diseased conditions, such as, embryonic development, cancer, quorum sensing, cardiac rhythm, which happen over many

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5 space and time scales (Glass, 2001). Interesting synchronization patterns are found in
6 ecological systems also. Here migration among population patches leads to broad-scale phase
7 synchronization with the peak population abundances remaining largely uncorrelated. This can
8 lead to the emergence of complex travelling-wave structures, which may be crucial for species
9 persistence (Blasius, *et al.*, 1999). Thus, the over all collective behaviour in biological systems is
10 an emergent property of the processes occurring at different scales.
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16 We have shown, with a simple model, that much of the smaller and larger scale
17 information of the spatial pattern and collective dynamics may be lost or remain unresolved
18 due to the coarseness or granularity of the measurement schemes. The intrinsic resolution
19 scale that decides the reliability of measurement can lead to incorrect and ambiguous
20 inferences about the system behaviour and can influence the predictive capability of the
21 experiments, at other scales. Similar problems have been discussed even in theoretical
22 methods where detecting modularity in social, biological and physical networks to study
23 community structures are limited by the detection algorithm (Fortunato and Barthe'lemy,
24 2007).
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32 To address this, modellers are developing software tools and mathematical approaches
33 to integrate data and models from micro-scales to macro-scales in a seamless fashion. Such
34 multi-scale models are essential if we are to produce quantitative, predictive models of
35 complex biological behaviours. In experiments, recent advances in measurement technology,
36 such as, large-scale gene expression, protein profiling, high throughput micro-scale imaging
37 and ultra-fast temporal measurements, along with intra-cellular measurements at single cell
38 level in a population (Auer, *et al.*, 2007; Rosenfeld, *et al.*, 2005; Golding, *et al.*, 2005) have not
39 only significantly aided the concurrent study of large number of interacting components
40 underlying cellular functions, but are also allowing observations to be made at milli-, micro-
41 and nano-meter space scale to study biological processes as it unfolds. As is shown in Fig.1,
42 this would certainly improve mining of information at different scales of resolution and
43 complexity, and lead to a much deeper understanding of the physiological functions and
44 pathogenesis in multi-cell ensembles, for both basic and applied sciences.
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Response to the Referee's comments on the manuscript (JBSC-D-07-00054)

“Measuring collective behaviour of multi-scale ensembles: Role of space-time scales”
by S. Rajesh and Somdatta Sinha

We thank the referee for carefully going through our paper and pointing out the discrepancies. We are also happy that he found our motivation for the work and the results interesting.

Based on the suggestions, we elaborate the corrections made below –

1) *The introduction is interesting but a bit long (more than 4 pages), in comparison with the discussion, which is rather brief (1 page). This creates a kind of imbalance within the manuscript. Perhaps part of the material could be moved from the Introduction to the Discussion? This is only a suggestion, and I leave it to the authors to decide what is best for making their point.*

We tried hard to delete or transfer text from the Introduction. But it is breaking the flow of the reasoning, and so we decided not to reduce it. The Discussion now is a bit longer due to insertion of text based on the last comment. We hope it is OK.

.....

2) *Page 5, last line: “Bertam” should read “Bertram”.* Correction incorporated.

.....

3) *Page 6, last line at bottom: Some words seem to be missing in the sentence “It was shown that different types...”*

The missing words have been inserted and the sentence now reads as – “It was shown that this ring of cells exhibits different types of global collective dynamics”

.....

4) *In Fig. 4, the lettering of the 12 panels corresponds to what is indicated in the text on p. 12 but apparently not to what is indicated in the figure legend. There, a, b, c and d apparently refer to the four rows, from top to bottom. Thus, something is wrong in the way the panels are labeled (the letters a, b, c, d are used both for the rows and for four of the panels) and referred to in the text or in the figure legend.*

The legend has been corrected.

.....

5) *On page 13, first line: the authors refer to “phase synchronization” but mention, on line 3, that “all the cells in the ring do not oscillate at the same phase...”. This apparent contradiction needs to be clarified.*

The sentences have been appropriately changed to incorporate the special features that are observed in this state. The text now read as follows –

“In Fig.4(g-i), for $N=50$, $e=0.72$, the ring of cells exhibits an interesting case of *phase synchronisation with phase slip*, where, even though the chaotic dynamics in each cell is completely suppressed, and they show stable periodic (Period 4) oscillations, but all the cells in the ring do not oscillate at the same phase, but are phase-locked to be in a synchronised state. There is a phase entrainment with a spatial

pattern of phase slips that allows the global dynamics of the ring of cells to be “two high peaks followed by two lower peaks” (see Rajesh, et al, 2007 for details).”

6) *The variety of patterns seen in Fig. 4 is closely associated with the chaotic nature of the oscillations that occur within each cell prior to coupling. I wonder (i) how large is the domain of chaos in parameter space, and (ii) whether there are also multiple modes of dynamic behaviour upon coupling the cells when the oscillations within each of them are periodic rather than chaotic, which might represent a more common situation.*

We have added a small paragraph in the beginning of Results section (Page 9), as this is a common question that may arise in the reader’s mind.

“It may be mentioned here that this pathway exhibits a wide range of dynamics—equilibrium, limit cycle, period-doubling, birhythmic, complex, and chaotic oscillations—with variation in parameters, and the complex and chaotic dynamics is observed in larger parameter space as the co-operativity of negative feedback (n) increases (Suguna, et al, 1999). Earlier study (Suguna and Sinha, 2005) with coupled cells at different dynamics regimes showed that – (i) the global dynamics in the coupled cells are fully synchronised, with the same periodicity and amplitude as the individual oscillatory cells. Thus, the collective dynamics of the coupled cell system is identical to that of a single cell in this regime; and (ii) cells at birhythmic states synchronized to the faster oscillation type for larger lattice size.”

7) *Page 16, section 3.3b, line 2: do the authors mean “on the sampling rate...” or, rather, “by the sampling rate...” ?*

This change has been made.

A final remark pertains to an article entitled “Complex dynamics and phase synchronization in spatially extended ecological systems ». In this paper published in 1999 in Nature (vol. 399, pp. 354-9), Blasius et al consider a three-variable chaotic system with spatial coupling leading to synchronization when the coupling strength is sufficiently large. Although this reference bears on ecological rather than cellular systems. the authors might consider the suitability of including a reference to it.

We have included a few lines in Discussion on this (page 21) -

“Interesting synchronization patterns are found in ecological systems also. Here migration among population patches leads to broad-scale phase synchronization with the peak population abundances remaining largely uncorrelated. This can lead to the emergence of complex travelling-wave structures, which may be crucial for species persistence (Blasius, *et al.*, 1999). Thus, the over all collective behaviour in biological systems is an emergent property of the processes occurring at different scales.”

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Measuring Collective Behaviour of Multi-Cellular Ensembles:

Role of Space-Time Scales

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