# Spatial Attractors in Aggregation Patterns of *Dictyostelium discoideum*

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Chemotactic cell motion in aggregation patterns of the slime mould *Dictyostelium discoideum* is analyzed by a computerized cross-correlation method. In this excitable medium the movement of amoebae is selforganized by signals of cAMP that propagate as rotating spirals or expanding concentric circles (target patterns). A vortex-like rotation of cells is found close to the core of spiral waves, with a maximum velocity of 15 μm/min. Cell motion and spiral tip orbiting follow an opposite sense of rotation. The calculation of streamlines of the most probable trajectories reveals the exsitence of a closed curve (radius ≈ 130 μm) corresponding to the boundary of the spiral core. This spatial-limit cycle attracts the amoebae on rotational paths and leads to the formation of a cell-free disk in the centre of the pattern. In contrast, pacemakers of target patterns organize cell movement in a radial, star-shaped motion, leading directly to the formation of central mounds.

## Introduction

The slime mould *Dictyostelium discoideum* is well suited for the study of cellular communication and a widely investigated example of selforganizing nonlinear systems. Single amoebae of this myxomycete aggregate to form a pseudoplasmodium. Subsequently, they differentiate to form a fruiting body bearing spores (Loomis, 1982). The depletion of their food source serves as signal to trigger the onset of the corresponding developmental cycle. The aggregation is coordinated and organized by traveling waves of cyclic adenosinemonophosphate (cAMP) (Tomchik and Devreotes, 1981). During this phase the majority of the population rests in an excitable state, while other cell groups serve as periodic pacemakers by producing cAMP according to an oscillatory mechanism. By means of diffusion their neighborhood becomes excited and relays the biochemical signal. Propagating waves result which show, in general, the geometry of concentric circles (target patterns) or rotating spirals (Fig. 1). These structures are usually investigated by darkfield-photography (Siegert and Weijer, 1989; Foerster *et al.,* 1990).

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The aggregation patterns formed in this living excitable system are similar to patterns observed in the chemical Belousov-Zhabotinsky (BZ) reaction (Winfree, 1972), during the oxidation of carbonmonoxyde on platinum crystals (Jakubith *et al.,* 1990), or propagation of calcium waves in Xenopus oocytes (Lechleiter *et al.,* 1991). The phenomenological similarity of these systems is reflected in their mathematical description, which is based on the general formalism of coupled reaction-diffusion equations (Tyson and Keener, 1988). The existence of additional chemotactic cell motion, following a non-diffusive mechanism, is an important particularity of the *Dictyostelium* system.

Martiel and Goldbeter (1987) proposed nonlinear kinetic rate laws describing oscillations and signal relay by *Dictyostelium* amoebae in well-stirred cell suspensions. These were extended by Tyson *et al.* (1989) to a reaction-diffusion model which is able to describe spatial pattern formation and implies the simplification of resting cells. In the real process amoebae are moving periodically and their motion interacts with the propagation of the chemical wave in a way which is not yet understood systematically (Alcantara and Monk, 1974). However, chemotaxis forces *Dictyostelium* cells to a movement in the direction of the strongest increase of cAMP-concentration (Fischer *et al.,* 1989). In an earlier study (Steinbock *et al.,* 1991) we found that the cell velocity in the periphery of

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wave patterns varies between nearly zero and 20–30 μm/min. The direction of the velocities of amoebae and chemical wave propagation are antiparallel; their magnitudes have a ratio of approximately 1:10. Probably only those spatial cAMP-gradients cause a chemotactic cell response that coincide with a local temporal increase in the cAMP-concentration.

This paper analyzes the chemotactic cell motion in the central region of spiral waves and target patterns in quantitative detail by applying a mutual-correlation method for the analysis of velocity fields. The method yields characteristic properties of attracting domains, which constitute a fascinating phenomenon of excitation patterns.

## Materials and Methods

### *(1) Experimental*

The cells of *Dictyostelium discoideum*, axenic strain AX-2, were cultivated on nutrient medium and harvested at a density of $5\times10^6$ cells/ml, washed three times with buffer and spread uniformly on an agar surface (containing 2 mM caffeine) in a Petri dish at a density of approximately $4\times10^5$ cells/cm$^2$. The dishes were stored in the dark at 21 °C for 4–6 hours. After this time the cell motion was detected with an inverse microscope (Zeiss IM 35) under bright-field illumination. The central region of the self-organizing structures (i.e. the spiral core or the pacemaker of a target pattern) was found by visual inspection. With a video camera (Hamamatsu C2400) and a time lapse video recorder (SONY EVT-801 CE) quick-motion movies (factor 25) were taken. A personal computer with an image acquisition card (Data-Translation, DT-2851) and an expanded memory card allows to store sequential image data of arbitrary size, image position and sampling speed. All subsequent calculations were performed on a Convex-C201 computer.

### *(2) Velocity analysis*

A specific space-resolved velocity analysis (Miike *et al.*, 1986; Hashimoto *et al.*, 1995), based on a mutual correlation method, turns out to be suitable for obtaining quantitative information about the cell motion. A moving cell, showing a characteristic contour (e.g. the cell boundary), causes temporal intensity changes at the traversed sites of recorded picture elements (pixels). If the contour is nearly constant in time, the intensity functions of neighboring pixels are shifted due to the cell motion by a retardation time $\tau_0$. This time shift is calculated by mutual-correlation functions $M_{0^k}(\tau)$ between the temporal brightness change of a central pixel at position 0 ($I_0(t) - I_0$) and that of its neighboring pixels at position $k$ ($I_k(t) - I_k$):

$$M_0^k(\tau) = \frac{1}{T}\int_{-T}^{T} (I_0(t) - \overline{I_0})\,(I_k(t+\tau) - \overline{I_k})\mathrm{d}t$$

($I_0$, $I_k$ denote the mean values of $I_0(t)$ and $I_k(t)$). For digitized time series the integral is reduced to summation terms. The fraction of the pixel distance and the retardation time $\tau_0$, when $M_{0^k}(\tau)$ reaches its maximum, yields the velocity $v_{0^k}$ in direction towards the neighboring pixel $k$. The maximum value of $M_{0^k}(\tau)$ is a measure for the probability that the cell was moving towards the site $k$. Due to necessary restrictions to be made, a considerable number of pixels is omitted in the calculations (Hashimoto *et al.*, 1995). Therefore, we commonly use spatial averages in appropriately selected areas. Spatial averages also suppress the component of stochastic cell motion, which is, as a charateristic property of amoebae, superposed on their chemotactic movement.

## Results

An example of a spiral-shaped cell aggregation pattern as obtained in the traditional dark-field illumination is presented in Fig. 1. In our velocity analysis microscopic sections of the pattern (bright-field illumination) are selected and recorded in digital image sequences. Microscopic quick-motion movies of the spiral core region ($0.39\times0.32$ mm$^2$) reveal a circulating cell movement. One large sector always shows a higher cell velocity than the remaining area. This sector circulates, but its sense of rotation is opposite to that of the individual amoebae.

Mutual correlation analysis allows a quantitative description of this phenomenon. Fig. 2A shows a velocity field determined in an $0.39\times0.32$ mm$^2$ area of the central region of a spiral. This area was digitized to an array of $512\times512$ pixels, each with

Fig. 1. Dark-field photography of a spiral wave in a two-dimensional layer of aggregating *Dictyostelium discoideum* cells. The pattern has a wave length of approximately 3 mm and a rotation period of 8 min.

256 possible grey levels. The velocity vectors were estimated from time series of 30 images corresponding to 120 s in the experiment. In Fig. 2A each vector is calculated by averaging the available information of the 51×51 surrounding pixels. Thus, the noise level due to the individual erratic motion of single amoebae is efficiently reduced. The vectors in Fig. 2A form a vortex like structure with a faster motion in the upper excited segment. Here, the highest velocity $v_{max}$ = 14.9 μm/min is detected. The slowest cell motion $v_{min}$ = 1.3 μm/min is found in the central part of the velocity field. In this region the chemical stimulus on the amoebae seems to be weaker than in the other parts of the wave pattern. The excited segment moves around the centre with a period of approximately 8 min.

In Fig. 2B temporal changes of the velocities are shown along a vertical cut ($y$-coordinate) through the centre of the velocity field in Fig. 2A. The changes $\Delta v(y)$ are calculated by substracting the local velocities at two different times $t_0$, $t_1$ from each other, where $t_0 - t_1 = 4$ min is approximately half of one spiral period. For small $y$ (the upper part of Fig. 2A) the velocities decrease, but for large $y$ they increase.

For a more detailed spatial analysis we calculated streamlines describing the most probable tracks of the amoebae. The applied algorithm constructs each streamline iteratively for arbitrary starting points, using only the angle of the velocities. We tested the reliability of the algorithm by applying it to gradient fields derived from simple

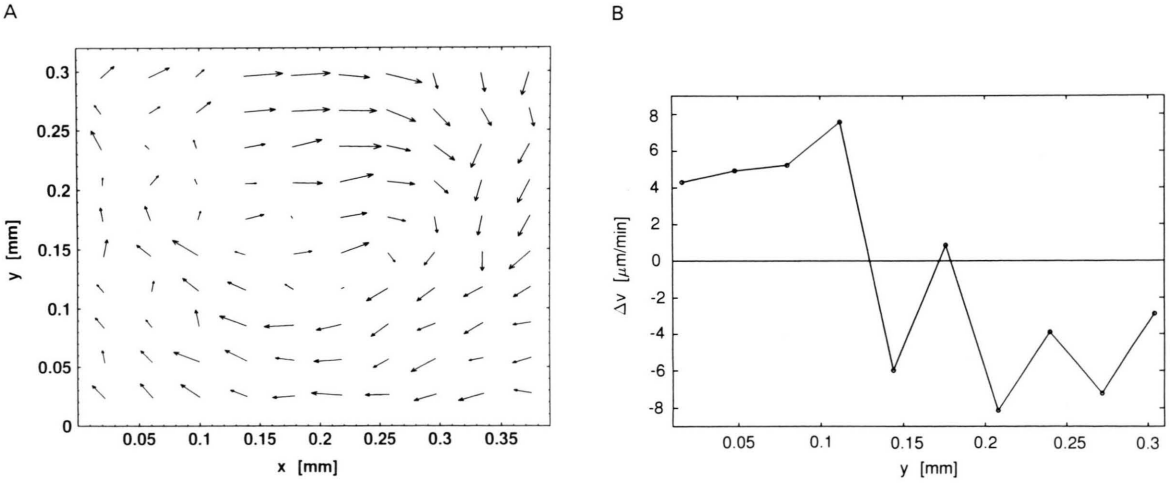


Fig. 2. (A) Velocity field of the chemotactic cell motion in the core region of a spiral wave pattern obtained by mutual-correlation analysis. The motion of the *Dictyostelium* amoebae forms a vortex-like structure. In this experiment the agar contains 2 mM caffeine. Analyzed area: 0.39×0.32 mm$^2$. (B) Changes of cell velocity $\Delta v$ over half a period of spiral rotation plotted against the $y$ coordinate passing through the spiral core. The variable $y$ corresponds to a vertical cut through the middle of the velocity field in Fig. 2A. The velocities are increasing for small and decreasing for large values of $y$. This behaviour is caused by an 180° rotation of the spiral tip.

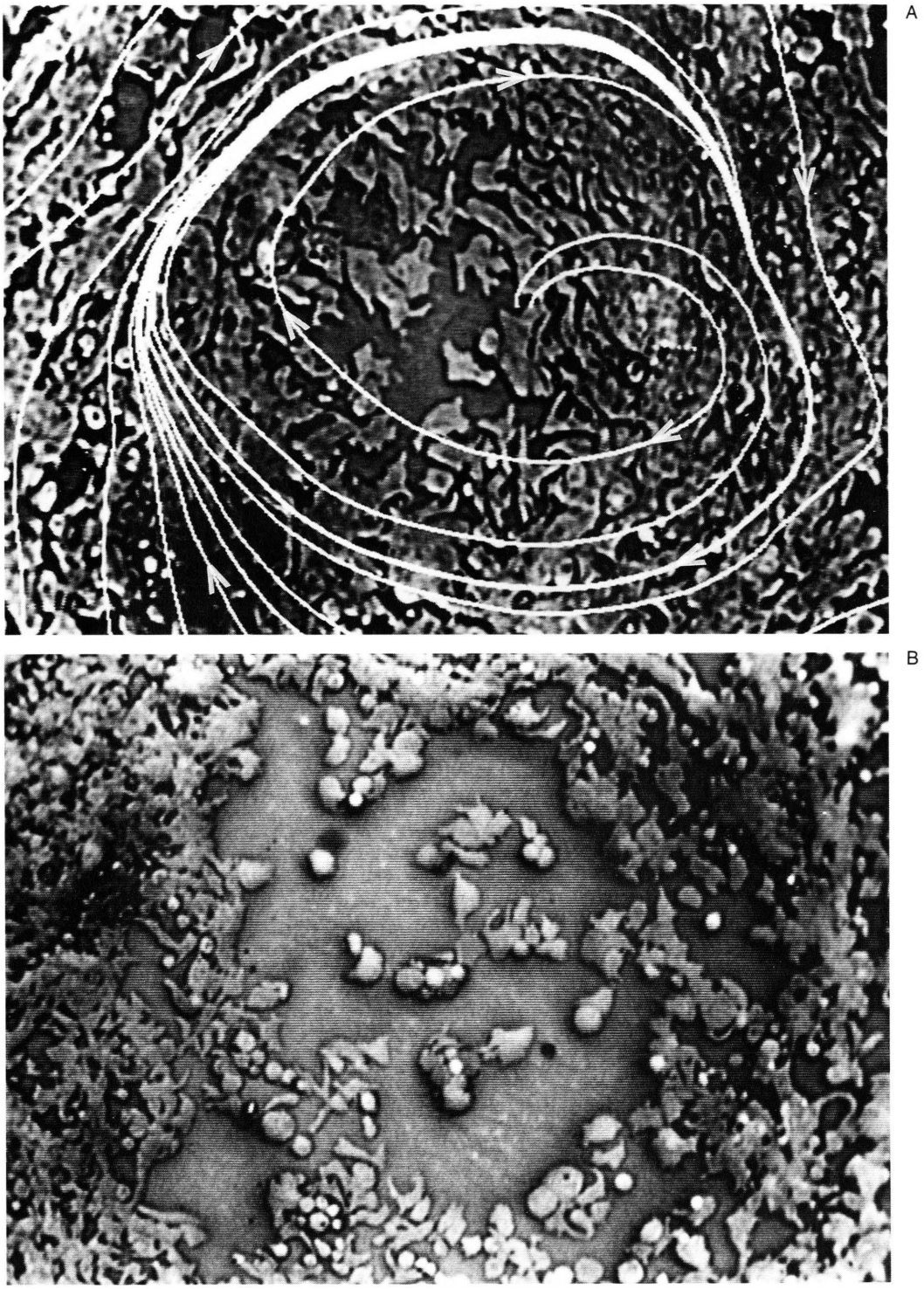


Fig. 3. (A) Streamlines calculated from the velocity field in Fig. 2A, describing the motion of the amoebae. The background shows one of the analyzed microscopic images of the core region. A closed curve seperates two different domains of cell movement. In the outer region, cells are aggregating in a circulating manner, in the inner region cells are led outwards on spiralling tracks. For this reason we call the closed curve of the streamline structure the spatial limit cycle of cell motion. (B) Microscopic image of the late stage of core region (2 mM caffeine). The image shows a $0.39 \times 0.32$ mm$^2$ section. A ring of high cell density encloses a region containing only a few amoebae. This hole is caused by cAMP-gradients in the spiral core leading cells slowly outwards.

mathematical formulae. The tests reveal good agreement between the reconstructed and theoretical curves. Fig. 3A gives a typical result of the analysis, based on the velocity field of Fig. 2. The streamlines are overlayed on a background showing one of the analyzed microscopic images of amoebae distribution.

It is remarkable that a closed streamline appears with the other trajectories nestling to this curve. The characteristic features of this movement are reminiscent of trajectories in phase space leading on a limit cycle. In a mathematically defined two-dimensional phase space representing solutions of a two-variable system of ordinary differential equations such a limit cycle is a closed path to which solutions are attracted asymptotically (Jordan and Smith, 1977). In our system, the area which is enclosed by the the spatial limit cycle has a size of 0.051 $mm^2$ and corresponds to the spiral core of the wave structure. A circle having the same area yields a core radius of approximately 0.13 mm. The cell movement along trajectories outside the closed curve possesses a dominating aggregative component. Inside the limit cycle the amoebae are led outside following spiral shaped tracks. This motion causes a decreasing cell density in the area inside the closed streamline. Fig. 3B shows a typical example of the resulting hole. The size of this hole covers the area of the observed limit cycles. Several spiral rotations later, we usually observed a decay of this arrangement into several new aggregation centres close to the hole. Dark-field images show that the pacemaker of the spiral wave in Fig. 1 vanishes and is replaced by three small target patterns, forming new individual centres.

By changing the caffeine concentration it is possible to control the core radius. Without added caffeine we find no detectable hole at all. With increasing caffeine concentration up to 5 mM the empty area is getting larger. One should notice that at this time the spiral shaped excitation wave circulates around a non-excitable region, without an effect on the basic spiral topology.

The central region of target patterns reveals significantly different chemotactic cell behaviour. In contrast to the vortex-like motion around spiral cores, we observed radial cell movement towards a singular point or very small domain. Fig. 4A shows a typical microscopic image (0.39×0.32 $mm^2$) of the centre of a target pattern. The correlation analysis of this region, performed with the same parameters as for the spiral core region, is given in the velocity-vector field of Fig. 4B. The velocities vary periodically, but reveal no significant angular dependence. Following the analogy between attractors in the phase plane and the chemotactic cell behaviour, we denote this kind of radial motion as spatial (star-shaped) nodes. Furthermore, the central part of target patterns shows no formation of a disk with low cell density:

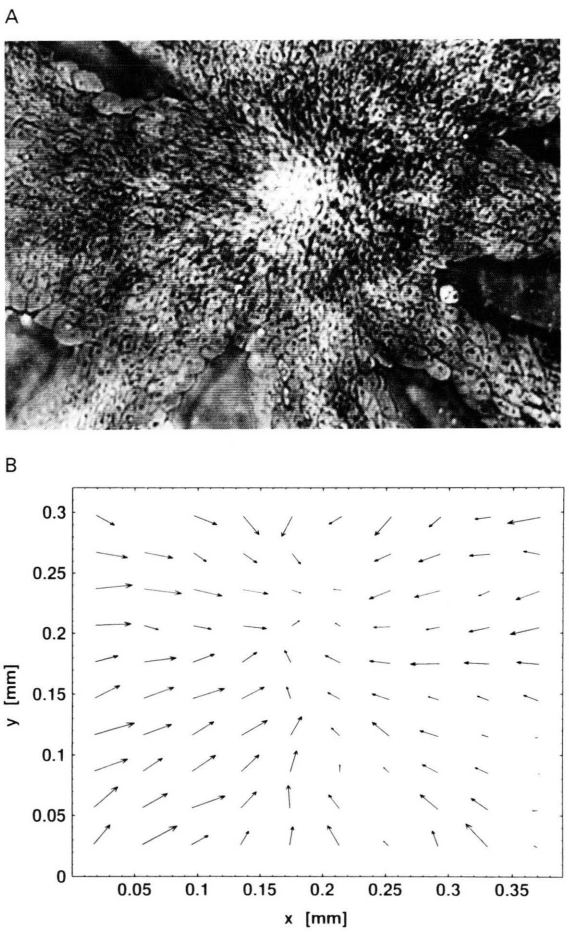


Fig. 4. (A) Microscopic image of the central region of a target pattern (0.39×0.32 $mm^2$; 2 mM caffeine). Quick-motion pictures show that the amoebae move periodically towards the central pacemaker. (B) Corresponding velocity field calculated from a 120 s sequence by mutual correlation analysis. The pacemaker of a target pattern controls cell aggregation like a star-shaped node attracting points in phase space.

the number of cells increases continuously and the initially two-dimensional cell layer is slowly transformed to a three-dimensional mound.

## Discussion and Conclusion

Our experimental results give quantitative information on the structure and dynamics of chemotaxis in the central region of spiral waves and target patterns. The analysis of pattern formation by measuring chemotactic cell response is a powerful tool, which reveals details that cannot be obtained with the common dark-field method. Furthermore, the complex chemotactic movement itself is a novel example documenting how structure formation occurs in nature.

The finding of a spatial limit cycle as the central attractor in spiral waves corresponds to the existence of a core in the central region of this aggregation process. The spiral tip spinning around this core leads to specific concentration gradients of cAMP, forcing the amoebae to the observed behaviour. Earlier investigations of spiral cores in the BZ reaction (Müller *et al.*, 1985; Plesser *et al.*, 1990) support this explanation and demonstrate the fascinating similarity of these systems: inside the chemical core analogous concentration gradients are oriented outwards. They change their direction continuously with increasing distance from the centre. Finally, in the periphery of spirals these gradients point exactly towards the core centre.

The formation of a central region of low cell density coincides with the emergence of a weakly or non-excitable disk. This process depends strongly on the caffeine concentration of the medium, which is an important parameter controlling the excitability of the system (Siegert and Weijer, 1989). Caffeine is known to inhibit the activation of the adenylate cyclase, while it has little effect on the cGMP formation which is associated with chemotaxis (Brenner and Thoms, 1984). Experiments in a light-sensitive version of the BZ reaction (Steinbock and Müller, 1992) have demonstrated an interesting analogy to the self-organized spiral cores of the slime mold system. Chemical spiral waves can rotate around laser-induced unexcitable spots ("artificial cores") that correspond to the central region of low cell density observed in the *Dictyostelium* system. The size of the laser spot changes the rotation period of the spiral, which is a characteristic constant value in homogeneous media. This correlation could explain the temporal evolution of the rotation frequency of spirals during the first two hours of *Dictyostelium* aggregation. Without caffeine Siegert and Weijer (1989) observed a nearly constant period (no holes). In the presence of caffeine (2 and 5 mM), however, they found the period to increase, because the diameter of the central hole influences the rotation period of the pattern just like in the BZ reaction.

In the central part of target patterns the chemotactic cell motion is controlled by a different spatial attractor (star-shaped node). In spite of the difference between spirals and target patterns the behaviour of amoebae in the periphery of these structures is the same. In conclusion, both types of attracting pacemakers guarantee an effective accumulation of cells, which is necessary for the formation of fruiting bodies.

It will be interesting to see, whether further numerical models, which include chemotactic transport, are able to simulate the described behaviour and elucidate the still open question of interaction between cell motion and wave propagation.

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