1.4

Mathematical Analysis of Cell Shape

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Cell motility involves translocation of the cell's centroid as well as changes or distortions in the cell's shape. Clues about the mechanism of cell movement may be obtained from information about its shape changes in time. The changes occur in multiple dimensions and can be highly periodic, however they may elude superficial observation. The techniques outlined in this contribution might help to reveal otherwise undetectable periodic shape changes.

The methods described here are appropriate for the analysis of spatio-temporal changes of closed shapes, such as cell outlines. They require an angular parametrization $z(\varphi, t)$ of the outline (for every angle φ and time t) refering to a suitably chosen point or area lying inside the cell. The methods are particularly suitable for qualitative and quantitative description of the lamellar dynamics of single motile cells with a distinct cell body and a flat periphery around it (e.g. human epidermal keratinocytes) but also for more amoeboid cells.

1 Representation of the outline

The parametrization of the outline depends on the particular problem to be analysed. In order to describe single motile amoeboid cells, the following parametrizations are suitable:
For an unbiased analysis of the spatio-temporal dynamics of the cell periphery, the representation of the cell outline has to be chosen independently of cell translocation. A parametrization $z(\varphi)$ in polar coordinates around the centroid of the cell seems a good choice. The location of the centroid is given by the two first order physical moments (m_{10}, m_{01}) of the cell:

$$m_{lk} := \frac{\int_Z x^l y^k f(x,y) dx dy}{\int_Z f(x,y) dx dy} \qquad 0 \leq l, k \tag{1}$$

where Z is the cell area and $f(x,y)$ the mass distribution within Z. For cells with distinct cell body and flat lamellae, the choice of two different constant weights for cell body and flat periphery, reflecting the mass difference between them (e.g. $f(x,y) = 1$ for the cell body, $f(x,y) = 1/20$ for the lamellae) reduces the bias on the location of the centroid due to widely extended lamellae. In case the cell body is indistinct, $f(x,y) \equiv 1$ may be chosen. If the cell has a flat lamella, but data on shape and location of the cell body are not easily available, its outline may be approximated by using information from the peripheral cell outline and the location of the cell body at the beginning of the time sequence. The cell body may be regarded as an inert

cell region that changes shape and position only if its outline comes into conflict with the peripheral cell outline because of global shape changes or cell translocation, for details see (Brosteanu 1994).

If the cell has an elongated shape, the bias due to deviation from a circular shape may be reduced by representing the cell outline $z(\varphi)$ for every angle φ as distance in the normal direction from the weighed momental ellipse of the cell; see Hinz & Brosteanu, I.2 this volume, Fig. 2. The weighed momental ellipse is defined by the centralized (around the centroid) second order physical moments, μ_{20}, μ_{02} and μ_{11},

$$E_Z := \left\{ (x,y)^T \mid \mu_{02}x^2 - 2\mu_{11}xy + \mu_{20}y^2 = 4\mu_{20}\mu_{02} - 4\mu_{11}^2 \right\}. \qquad (2)$$

This weighed momental ellipse has the same moments of second order as Z and approximates the shape and orientation of the cell body (cf. Alt *et al.* 1995).
A topographic line plot of the three-dimensional data set of $z(\varphi,t)$, with protrusions represented as elevated regions, gives a preliminary insight into the lamellar dynamics; see Hinz & Brosteanu (I.2 this volume, Fig. 3).

2 Angular Fourier analysis

The angular Fourier analysis of the outline

$$z(\varphi,t) = \frac{1}{2}a_0(t) + \sum_{k=1}^{\infty} a_k(t)\sin(k\varphi) + b_k(t)\cos(k\varphi) \qquad (3)$$

$$= \frac{1}{2}a_0(t) + \sum_{k=1}^{\infty} c_k(t)\cos(k\varphi - \psi_k(t)) \qquad (4)$$

provides a decomposition of the shape in symmetrical, k-modal components given by $\sin(k\varphi)$ and $\cos(k\varphi)$. The time course of the coefficients a_1 and b_1 (and perhaps a_2 and b_2) reflects the main shape changes. A plot of the path of a_k versus b_k reveals characteristic features such as rotating waves and standing pulsating waves (see Hinz & Brosteanu, I.2 this volume, Figs. 4C & F). In the case of rotating waves, Eqn. 4 with decomposition into shifted cosine functions may be used to describe the angular drift of the wave (by the shift coefficient ψ_k), the coefficient $c_k(t)$ quantifies the contribution of the k-modal component to the global cell shape at time t. Thus, $c_1(t) \gg c_k(t)$ $(k \neq 1)$ indicates situations with a single outstanding lamella, whereas $c_2(t) \gg c_k(t)$ $(k \neq 2)$ shows coexistence of two antipodal lamellae.

3 The Karhunen-Loève expansion

One particularly interesting way to extract the slaving modes from long sets of data is by reducing them by the Karhunen-Loève expansion (Fuchs *et al.* 1988, Friedrich & Uhl 1992, Krischer *et al.* 1993, Killich *et al.* 1994). For this purpose, one averages the distances $z(\varphi,t)$ from the origin (e.g. the cell centroid) to points on the two-dimensional boundary or margin (φ,z) in the angular direction φ for each of a series

of "snapshot" images of the cell, taken at frequent intervals of time. The actual distances $z(\varphi,t)$ may then be decomposed into the temporal averages $D(\varphi)$ and their actual deviations $d(\varphi,t)$, which in fact represent the shape of the cell's two-dimensional periphery:

$$z(\varphi,t) = D(\varphi) + d(\varphi,t). \tag{5}$$

The set of distances $d(\varphi_i,t_m), i = 1,2,\ldots,N$, forms a N-dimensional vector $\mathbf{d}_\varphi(t)$, where N is the total number of points on the cell's margin, measured at times t_m, $m = 1,2\ldots,M$. This vector $\mathbf{d}_\varphi(t)$ represents the temporal development of the cell's margin in the direction φ. The temporal developments of the margin in any two different directions φ_i and φ_j are explicitly correlated. The strength of this correlation is the scalar product of the vectors

$$\mathbf{d}_i(t) = (d(\varphi_i,t_1),\ldots,d(\varphi_i,t_M)) \quad and \quad \mathbf{d}_j(t) = (d(\varphi_j,t_1),\ldots,d(\varphi_j,t_M))$$

divided by M:

$$r_{ij} = \frac{1}{M}\cdot\mathbf{d}_i(t)\mathbf{d}_j(t) = \frac{1}{M}\cdot\sum_{m=1}^{M} d(\varphi_i,t_m)\cdot d(\varphi_j,t_m); \qquad i,j = 1,2,\ldots,N. \tag{6}$$

This allows one to obtain a so-called correlation matrix $R = (r_{ij})$. N eigenvalues $\epsilon_k, k = 1,2,\ldots,N$, belong to this matrix as well as N corresponding eigenvectors

$$\mathbf{s}_k(\varphi) = (s_k(\varphi_1),\ldots,s_k(\varphi_N)). \tag{7}$$

This set of N eigenvectors $\mathbf{s}_k(\varphi)$ represents an optimal set of discrete functions, which embody a description of temporal changes in the cell's shape.
A measure of the contribution of each eigenvector $\mathbf{s}_k(\varphi)$ to the actual shape of the cell's margin is the scalar product $c_k(t)$ of the eigenvector $\mathbf{s}_k(\varphi)$ and the vector

$$\mathbf{d}_m(\varphi) = (d(\varphi_1,t_m),\ldots,d(\varphi_N,t_m))$$

which is constructed from all deviations $d(\varphi_i,t_m), i = 1,2,\ldots,N$, at time t_m. If $c_k(t)$ is close to zero, the eigenvectors $\mathbf{s}_k(\varphi)$ and $\mathbf{d}_m(\varphi)$ are almost perpendicular. This circumstance indicates that the contribution of $\mathbf{s}_k(\varphi)$ to the actual shape of the cell margin is negligible, because the two-dimensional shape of the cell is the sum of the eigenvectors $\mathbf{s}_k(\varphi)$ weighted by $c_k(t)$. Otherwise, if the vectors $\mathbf{s}_k(\varphi)$ and $\mathbf{d}_m(\varphi)$ are nearly parallel, the $c_k(t)$ values will differ strongly from zero and it will be necessary to take into account the contribution of $\mathbf{s}_k(\varphi)$ to cell shape (from P. J. Plath in (Killich *et al.* 1994), see also (Vicker & Xiang, I.3 this volume)).

4 Angular-temporal autocorrelation

The correlation patterns of the outline $z(\varphi,t)$ are described by the autocorrelation function:

$$c(\alpha,\tau) := E_{\varphi t}\,[\,(z(\varphi,t) - E_{\varphi t}[z(\varphi,t)])\;\cdot\;(z(\varphi+\alpha,t+\tau) - E_{\varphi t}[z(\varphi,t)])\,] \tag{8}$$

where $E_{\varphi t}[\cdot]$ is the expectation value over φ and t. $c(\alpha,\tau)$ provides a measure for correlation of the outline in angular distance α ($0 \le \alpha < 2\pi$) and temporal distance $\tau \ge 0$. $c(0,0)$ is the variance of the outline extensions $z(\varphi,t)$. High values of the instantaneous angular correlation $c(\alpha,0)$ characterize the width of protrusions, whilst high values of the temporal correlation at the same angular position, $c(0,\tau)$,

characterize the duration of protrusions. Local maxima or elevated areas of $c(\alpha, \tau)$ at $\alpha > 0$ and $\tau > 0$ show typical spatio-temporal periods between protrusions.
The autocorrelation analysis reveals typical features of cell shape changes over time. Periodic or regular patterns as alternating standing waves or travelling waves are detected and quantified; see Hinz & Brosteanu (I.2 this volume, Fig. 4).

5 A comparison of the methods

Angular Fourier and Karhunen-Loève analyses are both based on the decomposition of cell shape using either sine and cosine functions or the shape eigenvectors, respectively. Both methods yield the temporal dynamics and relative contributions of each of the modal components which sum up to determine the shape of the cell periphery, and are suitable for qualitative comparison between different cells and between different "snapshots" of a cell. The Karhunen-Loève expansion, in its dependence on the eigenvectors of the individual cell, is of particular usefulness in the identification, quantification and comparison of the shape-relevant protrusion in case of cells with irregular shapes, where Fourier analysis shows no dominating component. On the other hand, Fourier analysis has the advantage that the k-modal components involved in the decomposition do not depend on the individual cell, and so Fourier analysis allows for quantitative comparison of the dominating modal components between cells. Autocorrelation analysis gathers information on the angular-temporal dynamics of the shape and enables not only detection of periodic patterns, but also quantification of the mean periodicity of shape changes.