Dynamic Pattern Formation on the Cell Cortex

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Abstract

Dynamic pattern formation at the junction between two cells has recently been observed experimentally and related to cell extrusion. This behaviour was re-created by modelling the 2-dimensional actomyosin cell cortex as an active fluid. The model qualitatively reproduced both the dynamic and stable pattern formation which had been experimentally observed in various physical systems. Phase diagrams for the analogous 1D model provided evidence dynamic behaviour occurred over a large range of parameter values. In the 2D model stress-induced actin breakdown was shown to give more connected chains of actin, whereas with a constant breakdown rate more isolated peaks tended to form.

1 Introduction

The interaction of actin and myosin can lead to spontaneous pattern formation on the cell cortex [1]. Recent research has allowed the observation of actin, myosin and cadherin concentrations at the interface between two epithelial cells, both on the zonula adherens and on the two-dimensional surface adjoined to it [2]. Both dynamic and static patterns were observed: static patterns involved multiple stationary peaks of high actin concentration, while dynamic behaviour was typified by peaks moving, merging, collapsing and new peaks spontaneously forming. [1] The qualitative behaviour of these

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[1] See Supplementary Material for videos. In particular the left and right images of V6 capture both behaviours.

patterns was shown to be related to whether or not the cell would be extruded from the epithelial layer.

Many different models have been proposed for the macroscopic interaction of actin and myosin, but most agree in treating the system as an *active fluid*, that is, a fluid in which the local stress in an explicit function of the local concentration of one or more species. This is physically reasonable (as in the presence of myosin large actin molecules will spontaneously contract, increasing local stress), and models based on this hypothesis have been successfully applied to a diverse range of physical systems, including lammellipod behaviour in moving keratocytes [3,5] and muscle sarcomeres [6]. The spontaneous formation of patterns in one of these models was reported in [1], but this model was only in one dimension and the only pattern which was observed was a single, stable peak of high actin concentration. They did not observe the formation of stable, multipeak solutions or of dynamic solutions, the two most common behaviours observed experimentally. In the following we demonstrate that a natural extension to this model allows the qualitative reproduction of both these types of behaviour, in both one and two dimensions.

2   Development of Mathematical Model

The mathematical model developed was based upon the following qualitative understanding of the biological system (see Figure 1).

1. **Actin Network Condensation:** Actin molecules are large polymers composed of many monomers, and given the right conditions these monomers will spontaneously polymerise.

2. **Peak Formation:** In the presence of myosin actin molecules will spontaneously contract (it is this behaviour which all muscles systematically exploit.) Under the right conditions they may contract to form concentrated peaks which continually pull the local velocity field inward (this inward flux is balanced by the outward diffusion). These peaks may be stable or dynamic.

3. **Actin Turnover:** Large actin molecules only have a finite lifespan, and eventually break back down into monomers. It is unclear to what extent this breakdown is stress induced.
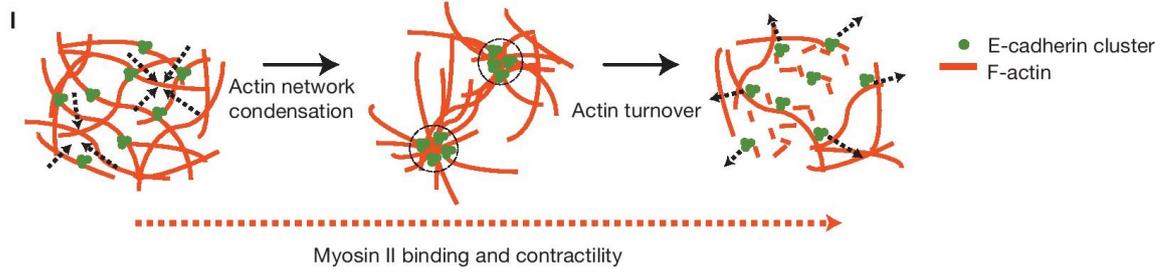
Figure 1: Qualitative Model for Actin-Myosin-Cadherin Behaviour on Cell Cortex. Source: [2]

It was natural to model the movement of actin, c, with a convection-diffusion-reaction equation, and to use a convection-diffusion equation for the conserved myosin, m:

$$c_t = c_{xx} - \text{Pe}c_t + \alpha(c_0 - c) \tag{1}$$

$$m_t = m_{xx} - \text{Pe}'m_t. \tag{2}$$

The velocity field at a given time was derived by first considering the following stress term (as in [1]):

$$\sigma = \eta\partial_x v + (\xi\Delta\mu)_0 \left(\frac{cm}{1+c}\right)$$

Here there are two independent contributors to stress: a viscous term ($\eta\partial_x v$) and an *active stress* term related to the concentration of actin and myosin (it is proportional at low concentrations, but at high concentrations of actin (for a *saturated* system) the increase in stress is limited.) This term was then combined with the force balance:

$$\partial_x \sigma = \gamma v.$$

After non-dimensionalisation, we get:

$$v = v_{xx} + \left(\frac{cm}{1+c}\right)_x. \tag{3}$$

Several alternatives to this model were conceived. The possibility of adding a third differential equation to describe the *actin monomer concentration* was considered: in

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this system the combined concentration of actin polymers and actin monomers would remain conserved. However, the diffusivity of actin monomers would be significantly higher than the diffusivity of the polymers, and this meant the modelled monomer concentration was approximately uniform. In this case the more complex model approximately simplified to the model suggested above, so it was not considered further.

Another alternative was to remove the myosin term completely, and assume a uniform background myosin concentration. To test this, the diffusivity of myosin was taken to be 4 times larger than actin [5] and the model above was run with this restriction. Under these conditions the myosin concentration was nearly uniform, and indeed there was no discernible difference between the case of uniform myosin and the model which accounted for these slight fluctuations. Thus for most analyses this simpler model was used:

$$c_t = c_{xx} - \mathrm{Pe}c_t + \alpha(c_0 - c) \tag{4}$$

$$v = v_{xx} + \left(\frac{c}{1+c}\right)_x. \tag{5}$$

The possibility of allowing the breakdown of actin to be stress-dependent was considered, and some of the qualitative effects of this in the two dimensional case are discussed below.

2.1 Two Dimensional Extension

This model was extended to two dimensions in a natural way. The convection-diffusion-reaction equation became:

$$c_t = \nabla^2 c - \mathrm{Pe}\nabla \cdot (c\mathbf{v}) + \alpha(c_0 - c) \tag{6}$$

The velocity field equation must be slightly extended to account for the occurrence of both *bulk* and *shear* viscosities in higher dimensions:

$$\mathbf{v} = \lambda\nabla^2\mathbf{v} + (1 - \lambda)\nabla(\nabla \cdot \mathbf{v}) + \nabla\left(\frac{c}{1+c}\right). \tag{7}$$

3 One Dimensional Numerical Modelling

Initially we ignored the source-consumption term, setting $\alpha = 0$, which reduced our model to the conservative one found in [1]. As found there, only two types of behaviour
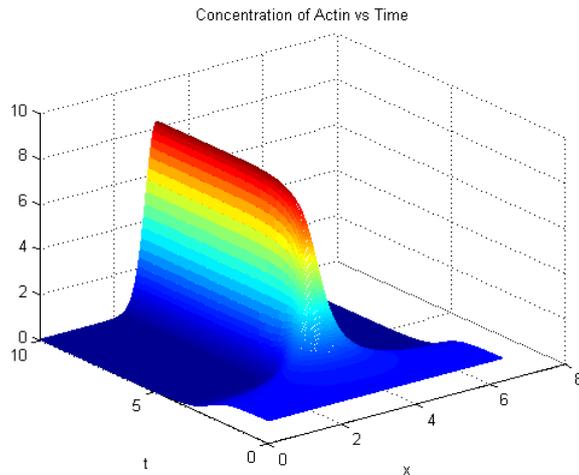
Figure 2: Single Stable Peak: concentration profile vs time

were observed: no peak would form, or a single stable peak would form (though for larger system multiple peaks would initially form, and these would merge, see Figures 2, 3 and 4). For the single stable peak, the net inward flux induced by the velocity field was exactly balanced by the diffusion away from the highly concentrated peaks.

A stability analysis for this system allowed the determination of the regions of the parameter-space in which patterns spontaneously formed (see [1]). However, the observations that dynamic behaviour was not possible and that multiple peaks were not stable (they eventually must join to form one large peak) couldn't be demonstrated analytically.

Next, the source consumption term was added to the model. Intuitively it was felt that this would allow for more dynamic behaviour, as it would allow new peaks to form in vacant regions and old peaks to break down (as observed experimentally). This was the case, and a few typical examples of some of the dynamic behaviour observed are given in Figures 6 and 7. What was perhaps more surprising was that the addition of the source-consumption term also *stabilised multiple peak solutions*, as can be seen in Figure 5. The exact reasons for this are still not fully understood.
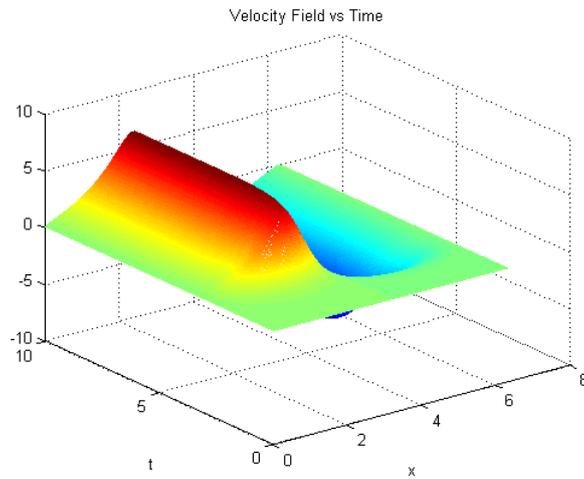
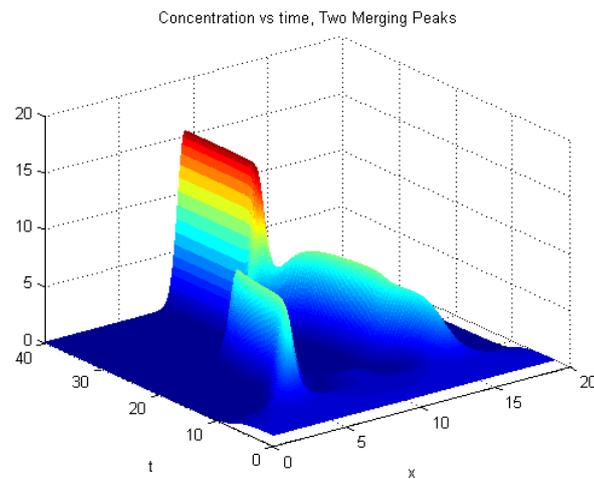Figure 3: Single Stable Peak: velocity profile vs time

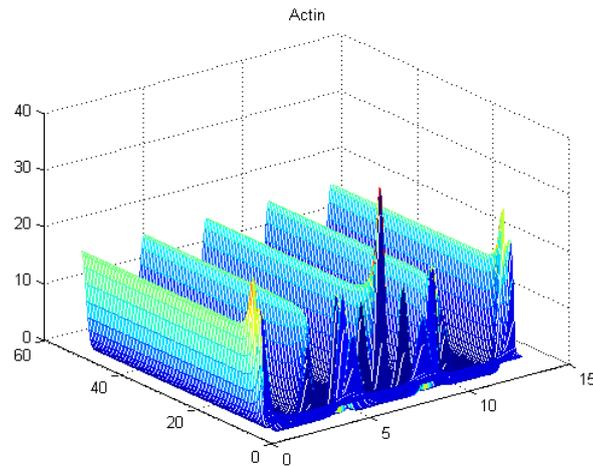Figure 4: Two Merging Peaks: concentration profile vs time

Figure 5: Accounting for actin breakdown and formation can allow stable multi-peak solutions

3.1 One Dimensional Phase Diagram

The biological system is in general dynamic, though occasionally multiple stable peaks may form. Thus in any reasonable model large portions of the available parameter-space should exhibit dynamic behaviour, and in order to test this, numerical phase diagrams were constructed for this system. Figure 8 shows a typical example, made from simulations of domain size 4π.

A few points should be observed here: firstly, the phase-diagram has a ring-like structure, with regions of dynamic behaviour sitting on the border between regions with different numbers of stable peaks. Here the system cannot decide which stable regime to sit in to, so it moves around dynamically. However we also see that there is a second regime of dynamic behaviour: for sufficiently high Peclet number and concentration we have large regions where the system doesn't stabilise. It is likely the biological system, which is usually dynamic, would sit in a region like this.
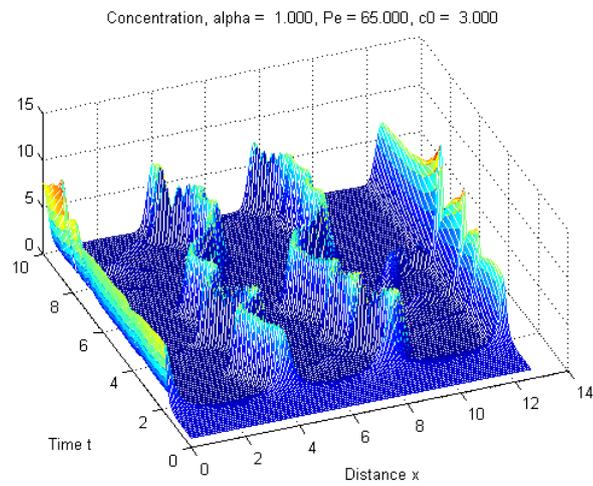
Concentration, alpha = 1.000, Pe = 65.000, c0 = 3.000

Figure 6: With actin turnover, peaks may move around seemingly randomly, while still remaining separate from each other

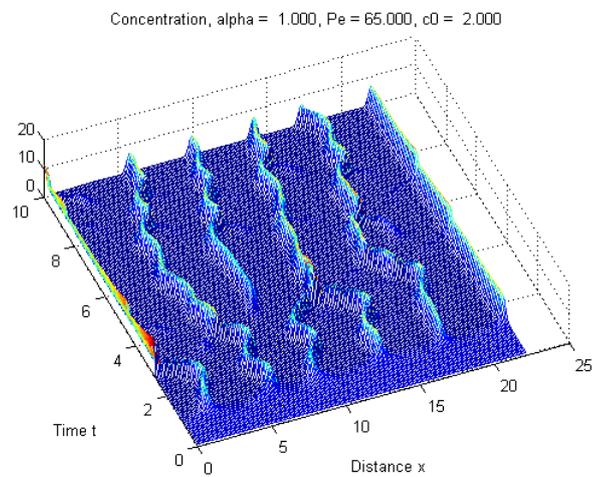Concentration, alpha = 1.000, Pe = 65.000, c0 = 2.000

Figure 7: Actin turnover also permits the formation of new peaks in vacant areas and the merging of two peaks into one
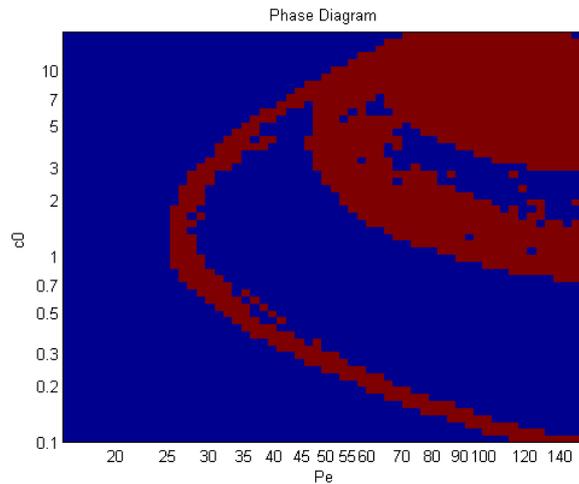
Figure 8: Numerically generated phase diagram of 1D system. $\alpha = 1$ throughout. Red regions denote dynamic behaviour, and blue stable behaviour.

4 Two Dimensional Numerical Modelling

Apart from the zonula adherens, the biological system of interest is a two dimensional surface along the junction between two cells, and so it is essential the proposed model gives 2D behaviour qualitatively similar to the physical system. A number of videos are available in the Supplementary Materials which show the behaviour of the physical system, but it can roughly be summarised as follows:

- The system is composed of many discrete clumps of actin and myosin.

- In some parameter domains (in particular when the actin turnover is reduced, as in the right hand side of video V6) these clumps remain stable and do not interact or move for long periods of time.

- In most parameter domains, clumps are more dynamic: they can be observed moving around, merging together, splitting apart and forming in formerly vacant regions.

The two-dimensional model described by equations (6) and (7) was modelled using a Lax-Friedrichs finite difference method (see Appendix A for a description of the numerical method). On a $4\pi \times 4\pi$ domain with periodic boundary conditions, as the Peclet number was increased the system demonstrated the three types of behaviour
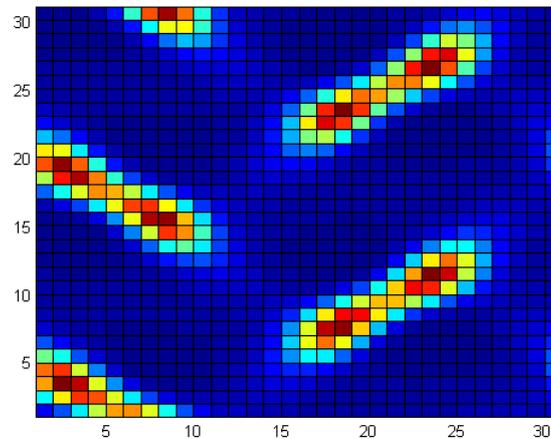
Figure 9: Unsymmetrical stable patterns in 2D

observed in the one dimensional case: no pattern formation, stable pattern formation and dynamic pattern formation. The stable patterns could form in a number of different geometric arrangements (see figures 9 and 10, along with videos V3 and V4), and for this reason there was no dynamic behaviour between different domains of stable arrangements, as occurred in the one dimensional case (i.e. the phase diagram wouldn't have the 1D case's ring-like structure.) However, the region of dynamic behaviour for higher Peclet numbers remained.

As can be seen in videos V1 and V5 in the Supplementary Materials, when the model behaved dynamically it was qualitatively similar to the real system. In the dynamic region peaks move around on their own accord, and regularly merge, split and form in previously vacant regions. Figure 11 shows a typical snapshot of this behaviour. Furthermore, in different parameter regimes (most notably with lower Peclet number and with less actin turnover) we see a variety of stable patterns, strongly reminiscent of the physical system's behaviour when actin turnover is limited (compare the two parts of V6, and the relationship between dynamic and stable models (e.g. V1 and V3) in the Supplementary Materials). This simple model thus seems to capture much of the qualitative behaviour of the system.
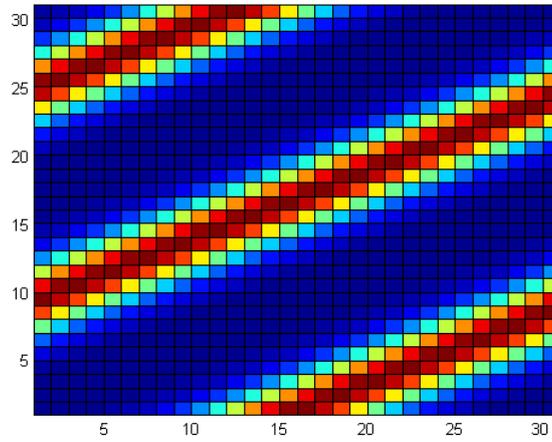
Figure 10: These stable peaks in 2D have effectively reduced to the 1D case
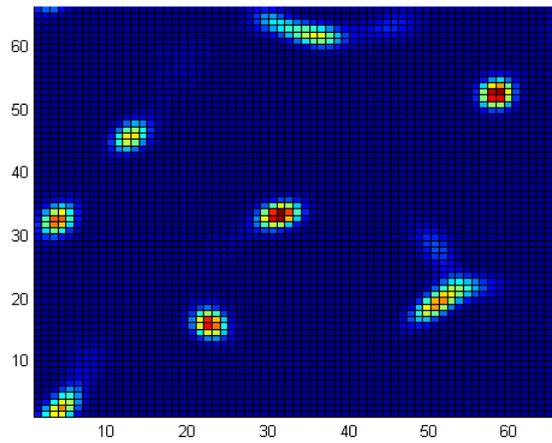

Figure 11: A snapshot of the dynamically moving, merging and splitting peaks in the model with constant breakdown rate, described by equations (6) and (7)

On the other hand, while in some experiments the peaks tend to be relatively isolated and circular (as in this model), often the peaks in the physical system can take more irregular shapes. Altering the convection-diffusion-reaction equation slightly, so that the rate constant governing the breakdown of actin is proportional to the actin concentration, we get a modification of (6):

$$c_t = \nabla^2 c - \text{Pe}\nabla \cdot (c\mathbf{v}) + \alpha(c_0^2 - c^2). \tag{8}$$

This change ultimately has the effect making the breakdown of the large actin polymers stress-dependent, which is quite reasonable physically. When this model is run the large, symmetric peaks become less stable, and peaks tend to string together in irregular, connected patterns, much like in the physical system (see video V2 in Supplementary Material, along with Figure 12). Note that this model does not artificially increase stress along any particular direction: at all times the clumps of actin pull symmetrically in all directions, which is reasonable given that in reality they form isotropic, tangled clumps. The breaking of symmetry associated with the chains of actin found in this model is thus an emergent phenomena caused by the strong attraction of actin clumps to their nearest neighbours.
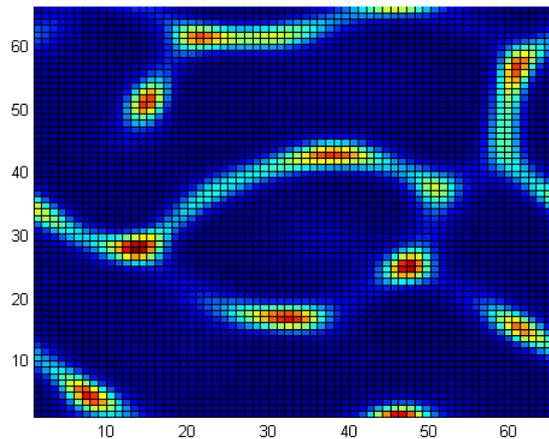
Figure 12: A snapshot of the dynamically moving, merging and splitting peaks in the model with concentration dependent breakdown rate, described by equations (8) and (7)

5   Conclusions

A simple 2D model of the cell cortex as an active fluid was able to reproduce much of the qualitative behaviour of the junction between two cells. Analysis of the one dimensional case demonstrated that dynamic pattern formation occurred for a large range of parameters, as required for the biological system. It also demonstrated that actin turnover was essential both for the formation of multiple stable peaks of actin and also for dynamic movement, creation and destruction of peaks. The qualitative behaviour of the 2D system was very similar to that observed experimentally, with both dynamic and stable patterns observed in different parameter regimes. The dynamic behaviour was also influenced by whether or not the rate of breakdown of actin was stress dependent: when it was stress dependent longer chains of actin tended to group together, more reminiscent of some observed systems. While this model provides qualitative validation of the idea that dynamic pattern formation arises spontaneously out of the active acto-myosin system, work is still required before it could give any quantitative predictions.

References

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Appendix A - 2D Numerical Method

Suppose we want to model the following convection/diffusion equation:

$$c_t = -Pe \cdot (vc)_x + c_{xx}. \tag{9}$$

If we decide to use the Lax Friedrichs finite difference method, we approximate the advection part of the PDE by the equations:

$$c_j^{n+1} = \frac{1}{2}\left(c_{j+1}^n + c_{j-1}^n\right) - Pe\Delta t \left(\frac{(vc)_{j+1}^n - (vc)_{j-1}^n}{2\Delta x}\right) \tag{10}$$

This method has significant numerical diffusion, as can be seen by rearranging it slightly:

$$c_j^{n+1} = c_j^n - Pe\Delta t \left(\frac{(vc)_{j+1}^n - (vc)_{j-1}^n}{2\Delta x}\right) + \left(\frac{(\Delta x)^2}{2\Delta t}\right)\Delta t \left(\frac{c_{j+1}^n - 2c_j^n + c_{j-1}^n}{(\Delta x)^2}\right) \tag{11}$$

Here we see we are modelling a convection-diffusion equation [2] with numerical diffusion equal to

$$D_{num} = \left(\frac{(\Delta x)^2}{2\Delta t}\right) \tag{12}$$

Thus we don't explicitly need to include a diffusion term - if we choose our parameters so $D_{num} \approx 1$, then we will have a valid model for (9).

Stability and Stepsize

From now, refer to Pe as the number in (9) and Pe_a as the *actual* Peclet number we are physically modelling. There is a discrepancy because (as can be shown from the non-dimensionalisation)

$$Pe_a = \frac{Pe}{D_{num}} \tag{13}$$

As mentioned before, we'd ideally like $D_{num} \approx 1$ so that $Pe = Pe_a$, but we won't assume this for now.

The CLF stability criterion approximately requires

$$Pe|v| < \frac{\Delta x}{\Delta t} \tag{14}$$

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[2]Accurate to second order accuracy, according to [4].

and substituting this into (13) and (12) gives

$$D_{num} = \frac{(\Delta x)^2}{2\Delta t} > \frac{Pe|v|\Delta x}{2} \tag{15}$$

and so

$$\frac{Pe}{D_{num}} = Pe_a < \frac{2}{|v|\Delta x} \tag{16}$$

or equivalently

$$\Delta x < \frac{2}{Pe_a|v|}. \tag{17}$$

This is an explicit limit on the size of Δx, and it is not unreasonable. Note that $|v| \sim f(x, t)$, and this condition must be satisifed at all times and at all points in space.

Higher Dimensions

In 2 dimensions the CFL stability condition changes to

$$Pe|v| < \frac{1}{\sqrt{2}}\frac{\Delta x}{\Delta t} \tag{18}$$

and the numerical diffusion changes to:

$$D_{num} = \frac{(\Delta x)^2}{4\Delta t}. \tag{19}$$

Combining these we have

$$D_{num} = \frac{(\Delta x)^2}{4\Delta t} > \frac{Pe|v|\Delta x}{2\sqrt{2}} \tag{20}$$

and so

$$\Delta x < \frac{2\sqrt{2}}{Pe_a|v|} \tag{21}$$

If we assume that $v_x = v_y$, then $|v| = \sqrt{2}v_x$ and so we get

$$\Delta x < \frac{2}{Pe_a v_x} \tag{22}$$

which is exactly the same condition as we had in the 1D case.

Practical Choices

For a given Pe, we should pick a value of $|v|$ which probably won't be exceeded (usually $|v| < 0.25$ is reasonable) and then use (21) to find the maximum stepsize, which should be used. Δt is then calculated from (19) so that $D_{num} \approx 1$.