Electronic Supplementary Material (ESI) for Integrative Biology.

Precisely parameterized experimental and computational models of tissue organization

Supplementary Materials: Description of the patterning algorithm

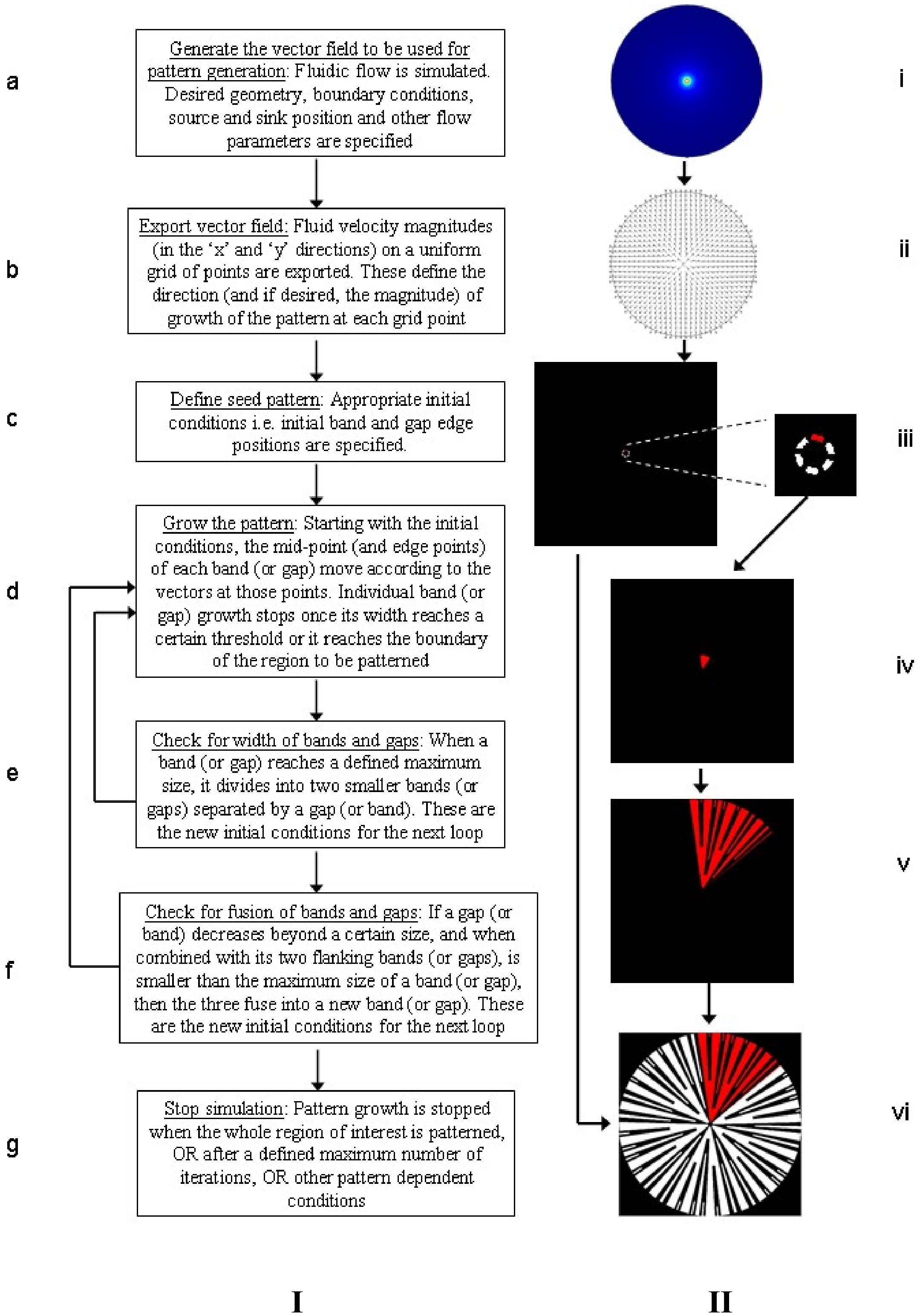


Fig. S1 A schematic description of the patterning algorithm

Description of the algorithm steps (refer to the schematic in Fig. S1 above; the steps are described on the left (I) and illustrated on the right (II))

The first step of our algorithm (a,b; i,ii in the Schematic) involves the generation of an appropriate vector field. One of the main proposals of the study is that tissue organization can be defined by a vector field generated by simulation of a physical process, such as a steady state fluid flow, or a physical setting, such as an electric or electro-magnetic field. The important consideration here is that the vector field developed by simulation of an otherwise irrelevant physical process or setting can have properties useful for design of complex model biological tissue representation, such as the local continuity and smoothness of cell orientation, combined with the potentially complex global character of the overall cell patterns. In this study, we generated a vector field through simulation of a two-dimensional incompressible isothermal fluid flow determined by the Navier-Stokes equations. The fluid flow can be defined on an arbitrary user-defined geometry, employing careful selection of boundary conditions for the pressure or velocity at each of the boundaries of the region to be patterned. As explained below and shown throughout the main text, we explored multiple types of boundary conditions leading to different flow fields. As the outputs of the simulation, fluid flow streamlines are generated, and the direction and magnitude of flow velocity at each point on the simulation grid is determined, comprising the vector field. In our typical simulations, only the direction of the vector is used while the amplitude of the vector is not required, and hence vector fields are assumed to have constant (unit) amplitude. However, it is possible to use the amplitude information in variations of the pattern generation algorithm. This field is then stored and can be used as a basic skeleton for both designing the masks for micro-contract printing and conducting simulations of the behavior of cell ensembles expected to result from the corresponding cell patterning.

The second step of the algorithm (c; iii in the Schematic) specifies the seed pattern, which lays the foundation of the ultimate pattern of alternating cell adhesive (bands) and non-adhesive (gaps) areas. More specifically, the bands and gaps of the seed pattern propagate through the region to be patterned according to the specifications of the underlying vector field (Fig. 1 a,b of main text). The initial conditions (bands and gaps) of the seed pattern are defined on the boundary of the vector field over which the vectors are directed into rather than out of the region to be patterned region, i.e., defined on the sources of the flow lines, including singular or point sources (in the latter cases, the seed pattern is defined on a circle surrounding the point source). It is important to note that the seed pattern can be combined with appropriate rules for band growth, branching and fusion given below, to generate the pattern of interest. Indeed, in the case of high divergence of flow lines (e.g., from a point source), one can often define a scaled down version of the seed pattern on the boundary surrounding the point source, controlling the number of bands and gaps rather than their absolute width values. As the widths expand during the seed pattern propagation, the required absolute values can be quickly reached. This property can allow a higher control of patterning around singular field sources as illustrated in Fig. 2 of the main text, and Figs. S3 and S4. Alternatively, in situations where band (gap) branching is not expected, one can define the seed pattern to have the appropriate final dimensions of the band (gap), e.g. in Fig. S4a.

The third step of the algorithm (d, iv in the Schematic) specifies propagation of the seed pattern through the region being patterned. As the seed pattern propagates, the cell adhesive and repellent segments extend to become bands and gaps, respectively. Specifically, the vector field defines the local direction and/or extent of translocation of the positions of the boundaries (end-points) and mid-points of bands and gaps, as shown in the Fig. S2 below. The transverse direction of the band (or gap) at the new mid-point is assumed to be perpendicular to the direction of motion (vector field) at the previous mid-point.

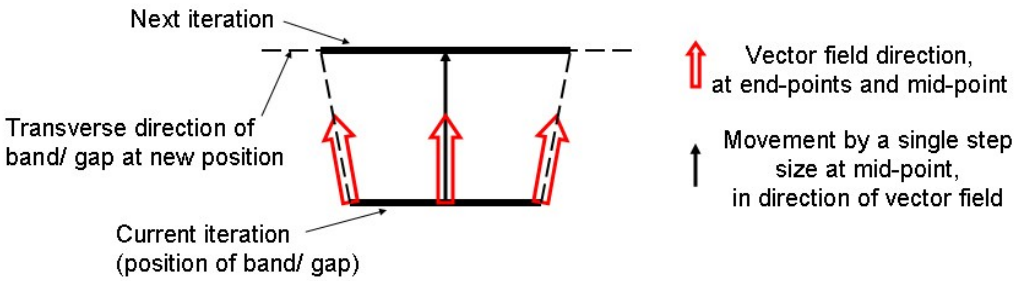


Fig. S2 Description of iterative step for band (or gap) propagation

The end-points of the band (or gap) for the next iterative step are determined by the intersection of the transverse direction vector of the band (or gap) at that iterative step and the vector directions corresponding to the end points at the current iterative step. The intersection points are calculated as the solution of a linear system of equations described below.

The following notation is used. The end-points of a band or gap at the current iteration are labeled as $(x_{n,1}, y_{n,1})$ and $(x_{n,2}, y_{n,2})$, and the mid-point is $(x_{n,3}, y_{n,3})$. The midpoint in the next iteration $(x_{n+1,3}, y_{n+1,3})$ can be calculated by moving by a fixed distance, say a unit step, in the direction of the vector field at the mid-point of the previous iteration. Using the same method, an estimate of the end-points in the next iteration $(\tilde{x}_{n+1,1}, \tilde{y}_{n+1,1})$ and $(\tilde{x}_{n+1,2}, \tilde{y}_{n+1,2})$ can be calculated. Since the actual end-points in the next iteration are calculated by the intersection of the transverse band (gap) direction and the vectors at the previous end-points, we can calculate them as follows:

$$(x_{n+1,1}, y_{n+1,1}) = inv(A_1)B_1 \quad and \quad (x_{n+1,2}, y_{n+1,2}) = inv(A_2)B_2, where$$

$$A_1 = \begin{bmatrix} \tilde{y}_{n+1,1} - y_{n,1} & -(\tilde{x}_{n+1,1} - x_{n,1}) \\ x_{n+1,3} - x_{n,3} & y_{n+1,3} - y_{n,3} \end{bmatrix} \quad \& \quad B_1 = \begin{bmatrix} x_{n,1} \times (\tilde{y}_{n+1,1} - y_{n,1}) - y_{n,1} \times (\tilde{x}_{n+1,1} - x_{n,1}) \\ x_{n+1,3} \times (x_{n+1,3} - x_{n,3}) + y_{n+1,3}(y_{n+1,3} - y_{n,3}) \end{bmatrix}$$

$$A_2 = \begin{bmatrix} \tilde{y}_{n+1,2} - y_{n,2} & -(\tilde{x}_{n+1,2} - x_{n,2}) \\ x_{n+1,3} - x_{n,3} & y_{n+1,3} - y_{n,3} \end{bmatrix} \quad \& \quad B_2 = \begin{bmatrix} x_{n,2} \times (\tilde{y}_{n+1,2} - y_{n,2}) - y_{n,2} \times (\tilde{x}_{n+1,2} - x_{n,2}) \\ x_{n+1,3} \times (x_{n+1,3} - x_{n,3}) + y_{n+1,3}(y_{n+1,3} - y_{n,3}) \end{bmatrix}$$

However, under certain special conditions, the determinant in the above-mentioned system of equations may be very close to zero (or equal to zero), resulting in abnormally high values for the coordinates of the intersection points. As an intuitive explanation, this may happen when the two ends of a band or gap are on extremely divergent flow streamlines. Under such conditions, the directional vector at one of the end-points may be almost parallel to the transverse direction vector of the band (or gap), resulting in an intersection at a very large distance from the previous point. In such a case, the algorithm uses the actual movement of the endpoint due to the vector field (rather than the intersection point with the transverse vector) for the next iteration.

If $\det(A_1) \cong 0 \quad or \quad \det(A_2) \cong 0$, then we define
$(x_{n+1,1}, y_{n+1,1}) = (\tilde{x}_{n+1,1}, \tilde{y}_{n+1,1})$, and $(x_{n+1,2}, y_{n+1,2}) = (\tilde{x}_{n+1,2}, \tilde{y}_{n+1,2})$

The fourth step of the algorithm (e,f; iv in the Schematic) involves the definition of the branching and fusion rules for bands and gaps. From the initial stages of the seed pattern propagation, the algorithm keeps track of the width of every band and gap. These widths can change if the local divergence of the field is not zero. Multiple rules can be formulated as to what might occur if the width of a band (or gap) exceeds its pre-specified maximum value, usually defined by the seed pattern, i.e., $L_{band} \leq 2 \times L_{band\_seed}$ (or $L_{gap} \leq 2 \times L_{gap\_seed}$), or in some cases, $L_{band} \leq L_{band\_seed}$ (or $L_{gap} \leq L_{gap\_seed}$). Some of these rules, explored in this study are listed below:

Rule 1) (Fig. 2C,D of the main text) Both bands and gaps are allowed to branch so that one band (gap) generates two new bands (gaps) and one new gap (band) of a predefined small width flanked by the two new bands (gaps). This typically occurs when L_{band} (L_{gap}) is equal to twice the width of the band (gap) zones in the seed pattern, L_{band_seed} (L_{gap_seed}). This branching can also occur when the L_{band} (L_{gap}) is equal to twice the maximum specified width of the band (gap) zones (when L_{band_seed} (L_{gap_seed}) are smaller than these maximum widths). This also allows variation of the band (gap) width from a predefined small width (following the gap (band) branching which leads to a birth of a new band (gap)) to $2 \times L_{band\_seed}$ ($2 \times L_{gap\_seed}$) just prior to branching.

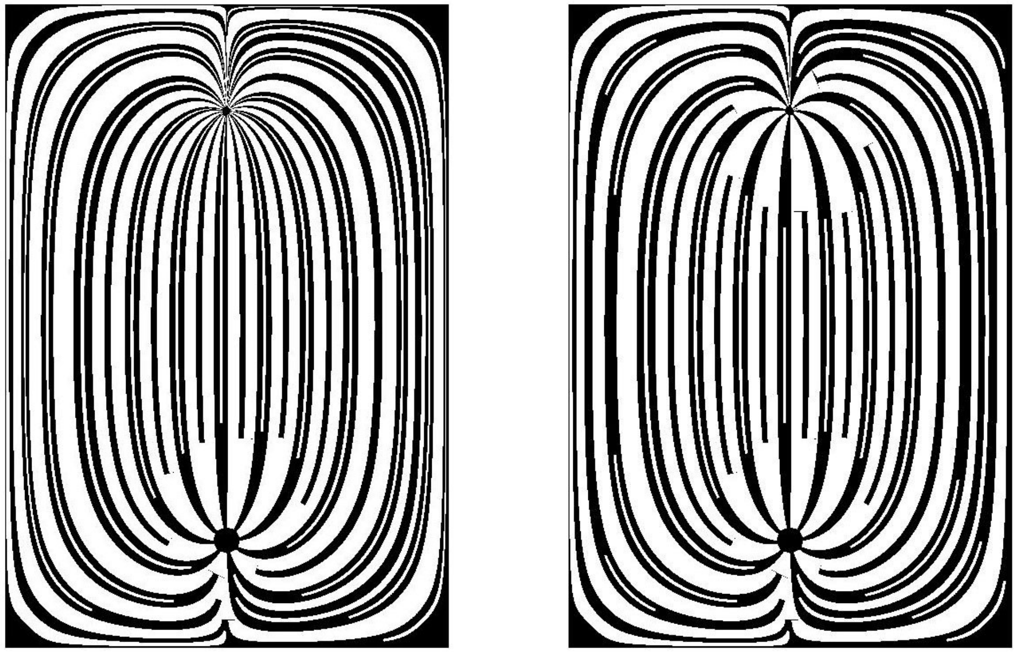
Rule 2) (Figs. 2F of the main text) Only bands (gaps) branch if $2 \times L_{band\_seed}$ ($2 \times L_{gap\_seed}$) values are reached, but gaps (bands) do not branch. The gaps (bands) are allowed to change their widths in a completely unconstrained fashion.

Rule 3) A Starburst pattern, such as the pattern used for experimental design, is shown in Fig. 2E of the main text. Gaps (bands) can be enforced to reach and then maintain the widths equal to the widths of the gaps (bands) in the seed pattern, without branching. In this case the divergence of the underlying vector field is followed during expansion of a newly born gap (band) up to L_{gap_seed} (L_{band_seed}). On the other hand, the bands (gaps) follow the underlying vector field and expand until they reach $2 \times L_{band\_seed}$ ($2 \times L_{gap\_seed}$) at which point the branching of the band (gap) is enforced, leading to formation of two new bands (gaps) and one new gap (band) of predefined small width.

Rule 4) The linear and concentric patterns used for experimental designs, as shown in Fig. 4A, C, correspond to a vector field with zero divergence. Accordingly, the bands (gaps) will maintain their initial width in the seed pattern, L_{band_seed} (L_{gap_seed}), and hence will not branch under either of the above 3 rules. The vector field merely guides the band (gap) growth direction in this situation.

Additional variants of the Rules 1-4 can be easily generated and implemented, underscoring the flexibility of the algorithm. Additional flexibility is afforded by varying the number and size of the initial gaps in the seed pattern (Figs. 2 C-F), as mentioned above.

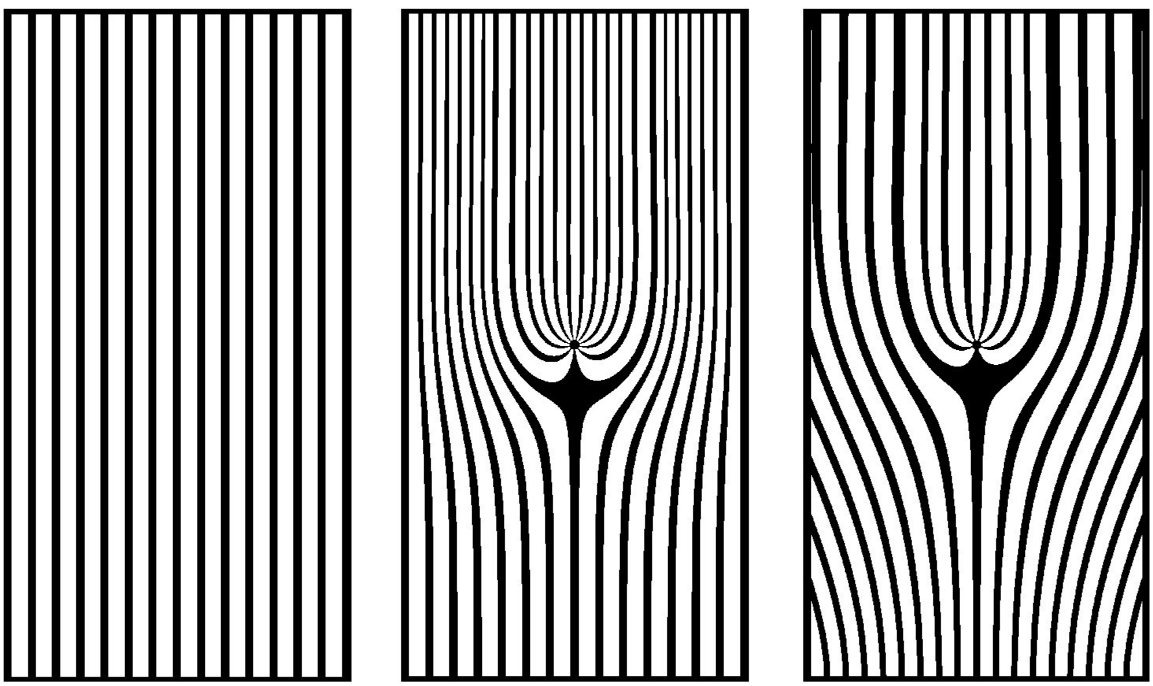
Although most of the cases considered involve branching of the bands and gaps, there are instances in which band or gap fusion may also be required. This might be the case in the presence of a field "sink", especially a single point sink. Rules similar to the rules for band (gap) branching can be formulated, specifying what happens if bands (gaps) reach widths smaller than pre-specified values in regions of negative divergence of the local vector field. Predominantly, the gap and two flanking bands (or vice-versa) that are eligible to fuse, arise from branching out of a band at an earlier iterative step, or have a common progenitor band (or gap) (see Fig. S3 for examples). Accordingly, for certain desired patterns, an additional constraint can be applied for the fusion step; whereby a gap and its flanking bands (or vice-versa) are allowed to fuse only if they have a common progenitor. To achieve this, the algorithm always maintains a history of the branching pattern of every band and gap.



a **b**

Figure. S3 *The algorithm allows for both, branching as well as fusion, of bands and gaps. A point source (large black circle at bottom of pattern) and a point sink (small black circle at top of pattern) exist in a confined rectangular space. The initial pattern generation (without fusion of bands or gaps) is shown in panel (**a**). This leads to progressive narrowing of both bands and gaps close to the sink. After fusion of bands and gaps is allowed, we obtain the pattern on the right **(b)**, which is intuitively the more appropriate pattern. It is also likely to help in efficient cell seeding on the bands because extremely thin gaps and bands are now fused with flanking bands or gaps respectively. Fusion was only allowed between bands and gaps with a common progenitor for this simulation.*

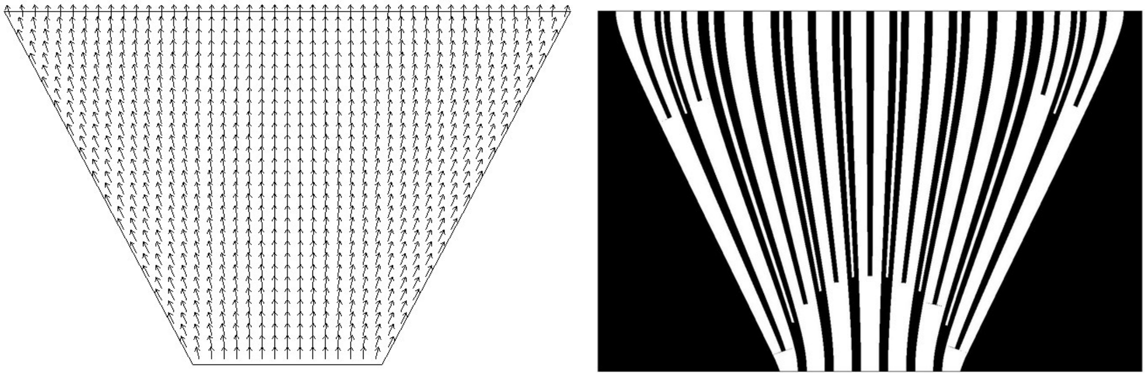
An interesting special case arises when there are multiple sources in the vector field (cf. Fig. S4a-c) . This can lead to fluidically independent regions, i.e., the regions in which all flow lines arise from separate sources, with separation boundaries between such regions. Although, the algorithm can be applied to different sources independently, as described above, a complication may arise. Namely, there may be bands or gaps which emanate from two different sources and may be situated too close to each other at the separation boundary. Since, in this situation, the seed pattern is effectively propagated independently in the fluidically separated regions, the separation boundary can be covered by an intervening gap (or band), which would be difficult to propagate using the rules as described above. This band (gap) can thus be deemed to be too thin or too wide. In such cases, it may be important to artificially increase or decrease the width of the intervening gap (or band) in an iterative fashion, during the specification of the initial conditions.



a b c

Figure. S4 *Variation of the boundary conditions as well as addition of an extra 'fluid' source in the center of a pattern can result in widely varying patterns.* ***(a)*** *A simple striped pattern results from fluid flow from the bottom of the patterned region;* ***(b)*** *and* ***(c)*** *correspond to fluid flow from the bottom of the patterned region along with an additional isotropic point fluid source at the center of the region.* ***(b)*** *corresponds to no-slip boundary conditions on the vertical edges, whereas* ***(c)*** *corresponds to neutral (i.e., no barrier) boundary conditions along these edges. Initial conditions were chosen to prevent branching of bands and gaps and to prevent two bands from different sources from being too close to each other at the separation boundary.*

The geometry of the patterned space can be quite diverse, which can also affect the results of the patterning process as illustrated in Fig. S5



a **b**

Figure. S5 *An asymmetric trapezoidal geometry leads to the conversion of a striped pattern from Fig. S4(a) to give rise to a new and qualitatively distinct flow* ***(a)*** *and band pattern* ***(b)****.*

The algorithm steps specify an iterative procedure that is stopped (g; vi in the Schematic) after the whole region of interest is patterned, or following the elapse of a specified number of maximum iterations, or when some other pattern-dependent conditions are met.