Supplementary Information

Computer Generated Patterns

A Monte Carlo based approach was used to produce the patterns of varying informational entropy in one axis. The general approach was to place spots into an 800x800 square x-y grid where the spot acceptance was constrained by a 1-dimensional (1D) potential. First a position was randomly chosen on the grid that was not within 16 points of the already occupied position. This excluded area was needed because the patterning tool used has a width of several micrometers, and placing spots too close would cause the tool to touch adjacent spots and distort or even remove them. A 1D biasing potential was applied to determine if the placement of a spot will be accepted. The 1D potentials were composed of sums of identical Gaussians (Eq. S1), evenly spaced across the pattern such that 8 Gaussians are centered within the grid and two sets of 8 are just outside (to reduce edge effects). A constant 0.01 was added to the potentials to ensure a non-zero probability that a spot would be placed at any point on the grid. The potentials were then normalized such that the maximum of the total potential function was equal to 1. Another random number was called and if it was less than the potential at that position, the Monte Carlo move was accepted, if not it was rejected. This process was repeated until 999 spots are placed on the grid. Ten different potentials were used in these experiments each one corresponding to different values for σ (8, 10, 15, 16.5, 18.5, 20, 30, 40, 55, 65) and different informational entropies (Fig. 1d).

$$Potential(x,\sigma) = C\left(\sum_{i=-8}^{15} 0.99e^{-(x-100*i-50)^2/2\sigma^2} + 0.01\right) \qquad \text{Eq. S1}$$

Here x is the axis perpendicular to the line direction, σ^2 is the variance of the Gaussians and C is a normalizing constant that insures that the potential has a maximum of 1. The computed pattern files were exported as coordinates in text files that were used for patterning as described below.

Computed Informational Entropy

For the ideal case with no excluded area, the informational entropy can be directly computed from the biasing potential. However, to account for the area exclusion, we calculated the entropy from computed normalized 1D histograms binned by x values. For each σ, patterns were generated and summed into until at least 80,000 patterns were made or until none of the bins were empty. These histograms where normalized such that the sum over all x was equal to 1. This is the effective probability that a spot will land at position x and was used to determine the entropy per spot from eq. 2. This number is then multiplied by 999 spots to get the total x entropy in the pattern. Fig 1D is a graph of predicted entropy and computed entropy versus σ.

Making Fibronectin Patterns

The fibronectin patterns were produced with a new microfluidic patterning tool, the NanoeNabler (Bioforce Nanosciences), using coordinates computed above. The NanoeNabler is based on a microfabricated surface patterning tool (SPT) that, like a quill-tip pen, allows small spots of an arbitrary liquid to be placed at predetermined locations on a surface. The spot size is in the range 2-30 µm, and is controlled by the operating parameters (e.g. the length of time the SPT is held in contact with the substrate). For the patterns described in this paper the SPT is loaded with a fragment of FN containing the primary cell binding site, type III repeats 7-10 of human FN (courtesy of Harold Erickson) at 1 mg/ml in spotting buffer (a proprietary buffer obtained from Bioforce). The patterns were made on Bellco indexed coverslips that had been modified with aminopropyltrimethoxysilane to have contact angles of ~65 to 80 degrees. The dimensions of the patterns were set by assigning a physical distance to the distance between two adjacent bins in the computed patterns, in the NanoeNabler software. Each pattern was made with two different bin spacings, 1.0 µm and 0.6 µm which generated the 25 µm and 15 µm patterns respectively. These correspond to overall pattern dimensions of 800x800 µm and 480x480 µm. After patterning the coverslips

were blocked with 3% bovine serum albumin for at least 30 minutes. Note that changing the size of the patterns does not change the amount of information in the pattern itself, but it does change the information density.

Cell Culture and Plating

Swiss 3T3 fibroblasts were maintained under standard culture conditions, and passaged every 2-3 days. For the experiments cells were treated as for passaging, but resuspended in serum free medium and plated on patterned coverslips. Serum free medium was used to reduce the adsorption of cell adhesive serum components to the substrates. The cells were incubated on the surfaces for 2 hours at 37 C and 5% CO_2, during which time they attached to the patterns and spread. Cells were then fixed and permeablized with 3% paraformaldehyde and 0.5% Triton X100.

Immunofluorescence and Microscopy

Cells were labeled using 1-1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI, Invitrogen), and a monoclonal antibody to the FN fragment (courtesy of Harold Erickson) was used to identify the patterns for orientation purposes. The antibody also stained intracellular FN and thus enhanced cell labeling. Focal adhesions were imaged by staining for a vinculin using a custom monoclonal antibody. The coverslips were then mounted onto a glass slide using FluorSave (EMD Biosciences, Inc., San Diego Ca). The samples were imaged using a Nikon epifluorescence microscope with a 20X objective, a 12-bit cooled CCD camera (CoolSnap HQ, Photometrics, Tuscon, AZ), and image acquisition software (OpenLab, Improvision, Lexington, MA). The aspect ratios of the cells in respect to the patterns were measured for all cells physically interacting with the patterns. The smallest box that contains a cell was drawn around each cell and the aspect ratio was determined by dividing length parallel to the pattern by the perpendicular length. The average aspect ratio was then plotted as a function of pattern entropy (Fig 3). Adobe Photoshop was used for all image-processing purposes.

Results for Figure 3.

Table 1. Pairwise Student's T-tests for 15 μm patterns. The numbers have been color coded to represent P <1%(red), <5% (green) and <10% (blue) chance that each set of data has the same mean.

σ	8	10	15	16.5	18.5	20	30	40	55	65
8	1	0.557	0.262	0.037	0.003	0.035	0.001	1E-6	1E-6	0
10	0.557	1	0.470	0.074	0.004	0.029	3E-4	1E-7	4E-7	0
15	0.262	0.470	1	0.327	0.060	0.150	0.007	3E-5	9E-5	4E-7
16.5	0.037	0.074	0.327	1	0.472	0.585	0.110	0.002	0.003	8E-6
18.5	0.003	0.004	0.060	0.472	1	0.931	0.175	0.004	0.013	6E-5
20	0.035	0.029	0.150	0.585	0.931	1	0.308	0.043	0.100	0.009
30	0.001	3E-4	0.007	0.110	0.175	0.308	1	0.264	0.476	0.053
40	1E-6	1E-7	3E-5	0.002	0.004	0.043	0.264	1	0.645	0.246
55	1E-6	4E-7	9E-5	0.003	0.013	0.100	0.476	0.645	1	0.079
65	0	0	4E-7	8E-6	6E-5	0.009	0.053	0.246	0.079	1

Table 2. Pairwise Student's T-tests for 25 µm patterns. The numbers have been color coded to represent P <1%(red), <5% (green) and <10% (blue) chance that each set of data has the same mean.

σ	8	10	15	16.5	20	30	40	55	65
8	1	0.031	0.816	0.807	0.850	0.814	0.091	0.159	0.218
10	0.031	1	0.101	0.024	0.104	0.064	0.848	0.947	0.840
15	0.816	0.101	1	0.960	0.956	0.962	0.363	0.304	0.351
16.5	0.807	0.024	0.960	1	0.981	0.996	0.221	0.159	0.197
20	0.850	0.104	0.956	0.981	1	0.985	0.249	0.295	0.360
30	0.814	0.064	0.962	0.996	0.985	1	0.204	0.234	0.297
40	0.091	0.848	0.363	0.221	0.249	0.204	1	0.928	0.991
55	0.159	0.947	0.304	0.159	0.295	0.234	0.928	1	0.932
65	0.218	0.840	0.351	0.197	0.360	0.297	0.991	0.932	1