# ALGORITHMIC FEATURE GENERATION FOR MICROSCALE TOPOGRAPHIES

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## Abstract

Using a parameterization of topographies, we design thousands of surface topographies spanning a wide range of parameter values. We use regression analysis to determine to what degree the parameters induce desired behavior of cells cultured on the topographies. Preliminary results indicate that there is significant correlation between the topographies and the cellular response.

## 1 Introduction

Cell behavior depends on multiple extracellular factors. One of these factors is the topography of the surface on which the cells are cultured [1,2]. We use high throughput screening (HTS) to identify topographies that induce desired cellular response and redesign topographies based on the results.

## 2 Generation of surface topographies

We design an initial collection of surface topographies by generating features. Features are topographies containing elements with a height of 10 µm arranged within an imaginary square with a size of 10x10 µm$^2$, 20x20 µm$^2$, or 28x28 µm$^2$. Features are built up using three types of microscale primitive shapes: circles, isosceles triangles (with one angle of 36° and two angles of 72°), and thin rectangles (3 µm thick). We choose these shapes, because by combining the primitive types we can generate different types of patterns: circles can be used to create large smooth areas, triangles can be used to create angles, and thin rectangles can be used to create stretched elements.

A feature is generated by first selecting uniformly at random one of the three possible feature sizes. Next parameter values are selected for the number of primitives used and the distribution over the different primitive types, the size of the primitives, and the standard deviation for the rotation of individual primitives. The distribution over the primitive types is selected such that all seven possible combinations of primitive types used (only circle primitives, only triangle primitives, only rectangle primitives, the three combinations of two primitive types, and the combination of all three primitive types) is as likely to be selected, and within a combination of primitive types used, each possible division is as likely to be

selected. Selections for all other parameter values are made uniformly at random from a range of possible values. Ranges for the parameter values depend on the size of the feature. See Table 1 for the parameter ranges for a feature of 20x20 µm$^2$.

| Parameter | Range |
|---|---|
| Number of primitives | 3 – 12 |
| Circle primitive diameter | 3 – 10 µm |
| Triangle primitive shortest side length | 3 - 8 µm |
| Rectangle primitive length | 3 -16 µm |
| Standard deviation for primitive rotation | 0 - 180° |

**Table 1:** Ranges for parameter values used for generation of a 20x20 µm$^2$ feature.

The orientation of a triangle or rectangle primitive is determined as follows: a triangle primitive is positioned with its sharp corner pointing to the right and a rectangle primitive is positioned horizontally, then the primitive is rotated by a number of degrees drawn from a normal distribution with mean 0° and standard deviation as determined during selection of the parameter values. The primitive is placed with the chosen orientation at a position where it is completely inside the feature, selected uniformly at random. Overlapping of primitives is allowed. See Figure 1 for four example features.
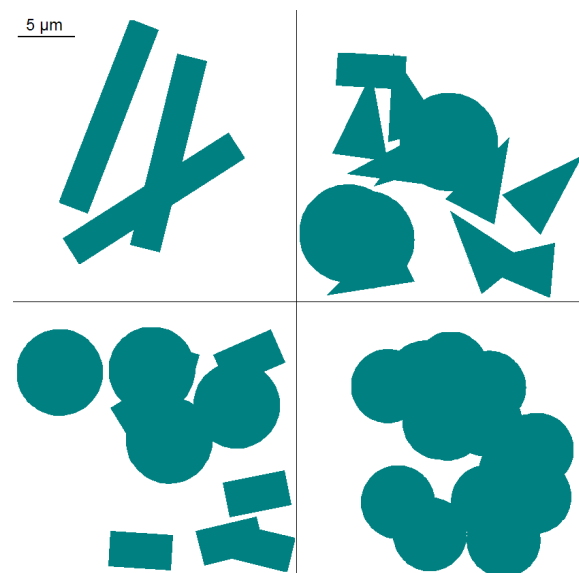


**Figure 1:** Four example features.

## 3 Practical construction

We create a polymer TopoChip containing thousands of 290x290 $\mu m^2$ units, separated by ridges. Each unit contains a feature, which is repeated to fill the unit. The part of the unit less than 5 $\mu m$ from the ridges is left empty. The lower left quadrant of the TopoChip is the same as the upper right quadrant, and the lower right quadrant is the same as the upper left quadrant. By this construction, each feature is present in two different units on the TopoChip; one unit on the top half and one on the bottom half. See Figure 2 for a picture of part of the mould used to fabricate the TopoChip.
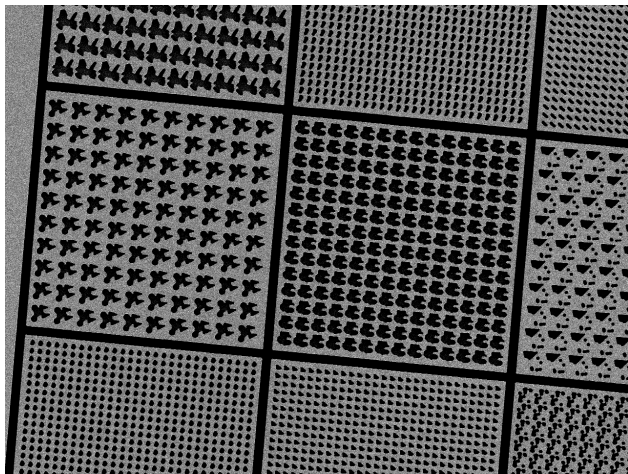


**Figure 2:** An image of part of the mould used to fabricate the TopoChip, showing several units. Each unit is filled with a repeated feature.

## 4 Measuring the cellular response

We seed cells on the TopoChip with a density of about 30-50 cells per unit and culture them on the topographies for six hours. The nuclei and the cytoplasm of the cells are stained using fluorescent dyes. We make fluorescence images of the nuclei and cytoplasm of the cells and use these images to measure the cellular response to the topographies.

## 5 Identification of influential parameters

In addition to the parameters used in the construction process of the features, we define extra parameters, for example the fraction of the feature that is covered by primitives. For each feature we compute the values of its extra parameters. We use stepwise regression analysis to identify a suitable model for the desired cellular response in terms of the most significant feature parameters. This model is used to determine promising topographies when new topographies are designed.

## 6 Preliminary results

We first investigate whether cells positioned on the same topography exhibit similar behavior. To do so, we make multiple copies of the TopoChip and compare cells in units which are on the same position on the TopoChip and thus contain the same topographies. Figure 3 shows representative fluorescence images of the same block of six units on two different copies of the TopoChip. Visual inspection of the images suggests that cells cultured on the same topography exhibit similar behavior. In the near future, we plan to use the fluorescence images of the cells to compute several cell characteristics. We will then apply principal component analysis using these characteristics to quantify the cellular response to the topographies.
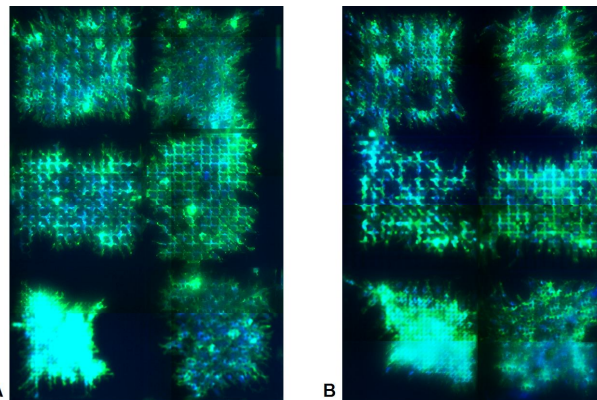


**Figure 3:** Fluorescence images of the same block of six units on two copies of the TopoChip. Fluorescent parts represent both cell nuclei and cell cytoplasm.

## 7 Future Work

Next to quantifying the cellular response using principal component analysis, we plan to combine real experiments (HTS) with virtual experiments, based on the results of the regression analysis. We will use methods from artificial intelligence such as genetic algorithms, to eventually identify topographies that maximally trigger desired cell behavior. This because real experiments are time-consuming. Combining real and virtual experiments can significantly improve convergence speed in the search for high quality topographies.

## References

[1] Dalby, M.J. et al. The control of human mesenchymal stem cell differentiation using nanoscale symmetry and disorder, *Nature materials* **6**:997-1003, 2007.

[2] Hatano, K. et al. Effect of surface roughness on proliferation and alkaline expression of rat calvarial cells cultured on polystyrene, *Bone* **25**:439-445, 1999.