CYTOO MICROPATTERN NETWORKS: FROM MODELING OF CELL SPREADING TO GLOBAL IMPROVEMENT OF siRNA SCREENING

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MEHTRICS aims to challenge the current limits of High-Throughput (HT) cell-based screening by combining HT-RNAi with an emerging new technology for normalizing cultured cell behaviors, namely micropatterns. The current CYTOO micropattern technology is optimized for short-term studies, i.e. within 1 cell cycle. To study longer-lasting biological processes, such as RNAi mediated silencing which typically requires at least 2-4 days for efficient knock-down, new micropattern networks are designed and characterized for their ability to normalize cell behavior over several cell cycles. To improve and predict the designs of these networks, mathematical models that recapitulate cell organization on extended micropattern networks are developed.

Since experimental designs required to run RNAi screens are among the most demanding of all HT/HC studies and encompass virtually all technical challenges also encountered in compound screens, we expect the proposed scope of activities to deliver the maximal potential for impactful innovation, widespread adoption and clear relevance for all major applications of HT/HC cell screening. The resulting new tools and methodologies are now incorporated into the respective commercial offerings of CYTOO and CENIX.

DESIGN OF MICROPATTERN NETWORKS USING COMPUTER ASSISTED MODELING

Design Of Micropattern Networks using Computer Assisted Modeling

$$E = \sigma A_{Cell} + \sum_{freearcs} \frac{EA}{L_i^{(0)}}\left(L_i - L_i^{(0)}\right)^2 + JC_{Cell} - \frac{W}{\rho + A_{Adhesive}} A_{adhesive}.$$

Figure 1: Left: time lapse images of a cell spreading on a V-shaped pattern. After the initial spread phase the arc like contour is reinforced indicated by the increasing radii of the fitted circles. Right: Spreading predicted by the cell-shape model which also shows a initial spread phase and reinforcement. Bottom: Energy function defined from time lapse data.

Cell spreading was modeled from individual micropatterns.

Simulation of cell behaviour : Results on Networks

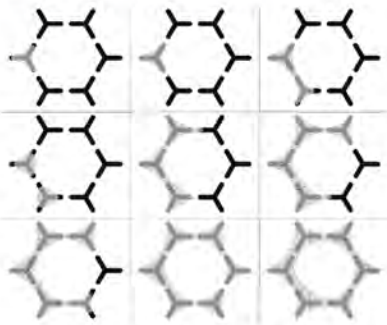


Figure 2: Time lapse model of a single cell spreading and dividing on six Y-shaped patterns arranged in a hexagon. Cell division takes place in periodic intervals.

Modelling helps to predict cell behavior on micropattern Networks designs.

Micropattern Networks Designs for *In Vitro* Tests

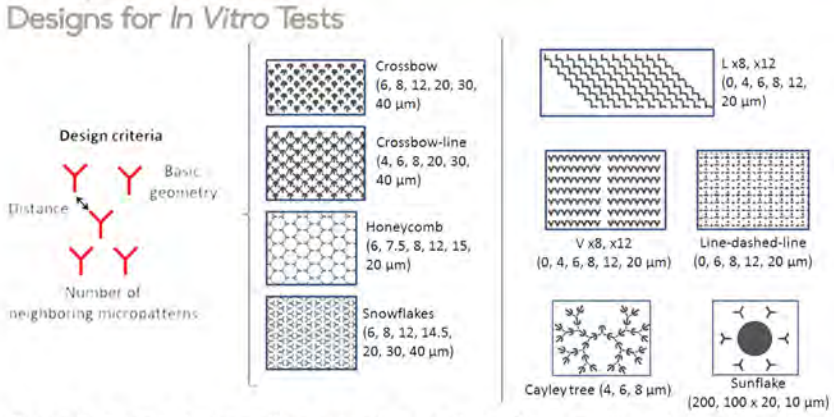


Figure 3: Range of micropattern network geometries. The key criteria for a micropattern network design take into account the geometry, size and spacing (Left). The possible designs are almost infinite: example of micropattern networks, distances between patterns are indicated (Right).

From more than 100 network geometries tested in vitro on 10 different cell lines, the best designs were selected to normalize cell behavior.

CELL BEHAVIOUR CHARACTERISATION ON MICROPATTERN NETWORKS

Cell behavior on Networks

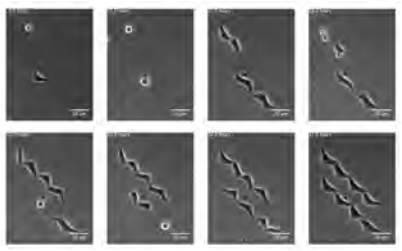
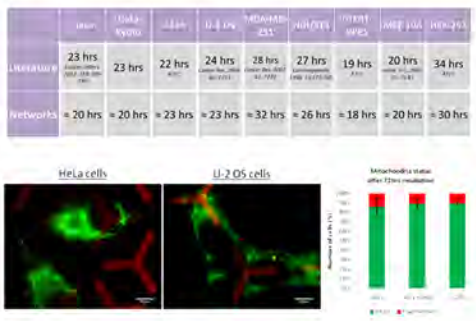


Figure 4: Time lapse analysis of HeLa kyoto cells seeded on a network composed of continuous "L" motifs. Pictures were taken every 30 minutes during 72 hours.

Micropattern Networks provides cell shape normalization over time.

Regards to Cell Health



Literature	23 hrs	23 hrs	22 hrs	24 hrs	28 hrs	27 hrs	19 hrs	20 hrs	34 hrs
Networks	≈ 20 hrs	≈ 20 hrs	≈ 23 hrs	≈ 23 hrs	≈ 32 hrs	≈ 26 hrs	≈ 18 hrs	≈ 20 hrs	≈ 30 hrs

Figure 5 Top: Comparision of cell doubling times on micropattern networks compared to classical 2D culture. Bottom: Evaluation of cell health at 72 hours on micropatterns networks by using mitochondria staining (Mitotracker Green).

Cell culture on networks does not modify doubling time and shows no mitochondrial stress.

Normalization of Cell Shape and Polarity

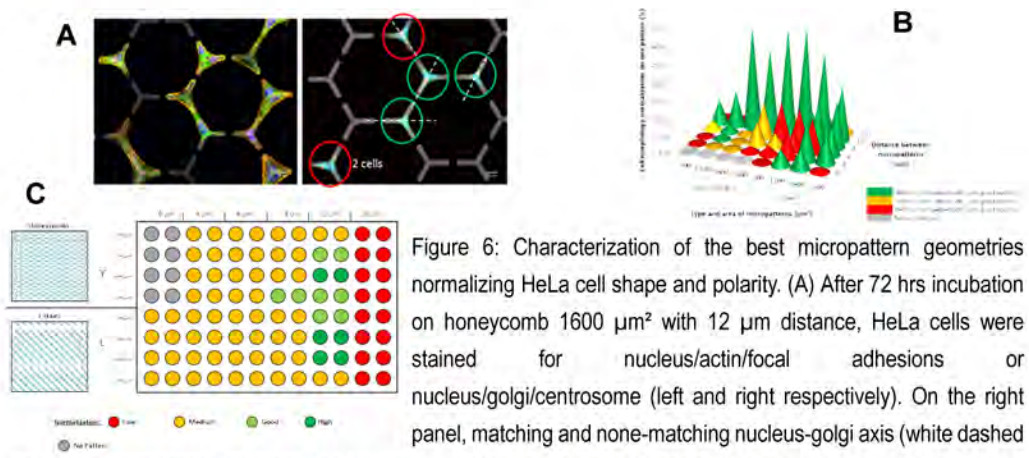


Figure 6: Characterization of the best micropattern geometries normalizing HeLa cell shape and polarity. (A) After 72 hrs incubation on honeycomb 1600 µm² with 12 µm distance, HeLa cells were stained for nucleus/actin/focal adhesions or nucleus/golgi/centrosome (left and right respectively). On the right panel, matching and none-matching nucleus-golgi axis (white dashed lines) versus micropattern branches (in gray) are shown in green and red circles respectively. (B) Rate of cell shape normalization on each pattern, n=283. (C) Results of HeLa cells characterization.

Network geometries with 12 µm distances are optimized to normalize the shape and polarity of more than 30% of micropatterned cells.

BENEFITS OF MICROPATTERN NETWORKS FOR siRNA APPLICATIONS

CENIX O-MP1™ Assay Overview

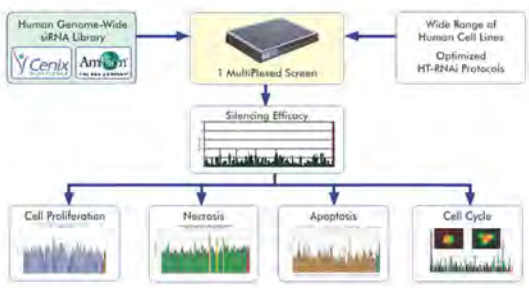


Figure 7: Description of the O-MP1™ test.

The Oncology MultiPlex-1™ (O-MP1™), monitors cellular proliferation plus key underlying processes, necrosis, apoptosis, and cell cycle progression within the same experimental run.

Networks Benefits on O-MP1™ assay

	Breast		Colon		Prostate	
	MCF7 (Non-Invasive)	MDA-MB-231 (Highly-Invasive)	HCT116 (Non-Invasive)	DLD1 (non-Invasive)	PC3 (Highly-Invasive)	DU145 (moderately-Invasive)
Proliferation						
Apoptosis						
Mitosis						
Other Phenotypes						
KnockDown						

Figure 8: Micropattern networks improve the O-MP1™ assay window in various cancer cell lines: cell proliferation (evaluated by nuclei segmentation), apoptosis (lamin A staining), mitosis (Edu staining), other phenotypes (multiple nuclear morphology parameters), siRNA efficiency). 6 different networks geometries were tested. Benefits on assay sensitivity are summarized as high (Green), good (Light Green), no benefit (White), and less sensitive (Red). **Independant results showed that Micropattern Networks improve the sensitivity of more than 40% of endpoints.**

Networks Benefits on O-MP1™ assay

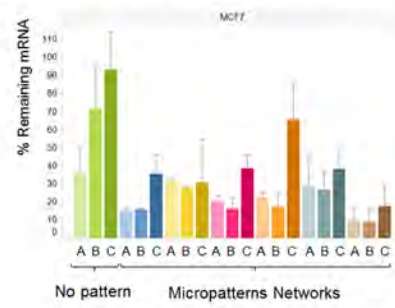


Figure 9: Example of micropattern networks effects on siRNA mediated knockdown. Three siRNAs with varying potency (A), (B), (C) were tested in MCF7 breast adenocarcinoma cell line. Colours represent 6 different types of micropattern network designs. All data were normalized to samples treated with negative control siRNA (100%).

As an example, siRNA efficiency can be increased up to 20% - 70% compared to classical 2D cell culture.