

Application of Adhesive Micropatterns in HCS improves sensitivity for drug effect detection and provides significant data from only 50 cells.

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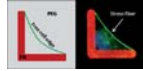
Abstract

To illustrate the potential of micropatterns for cell phenotyping in high throughput approaches, we conducted a series of assays with 4 drugs targeting directly or indirectly the actin cytoskeleton: Nocodazole, Blebbistatin, the ROCK inhibitor Y27632 and Cytochalasin D. Using automated image acquisition and analysis procedures, we performed dose/response assays on micropatterns in 96-well microplate format and showed that drug impact on cells can be efficiently quantified **even at doses one hundred times less** than that commonly used in research laboratory protocols. We also demonstrate that statistically significant results can be obtained with **very few cells (50)**, thus increasing throughput in High Content Analysis. Finally, we introduce the concept of the **Reference Cell™**, a cell-specific map that can be used as a standard for comparing and revealing intracellular differences after different treatment conditions. In conclusion, we demonstrate that micropatterns offer a clear advantage over conventionally grown cells for the detection, description and quantification of phenotypic effects when screening drugs.

Background

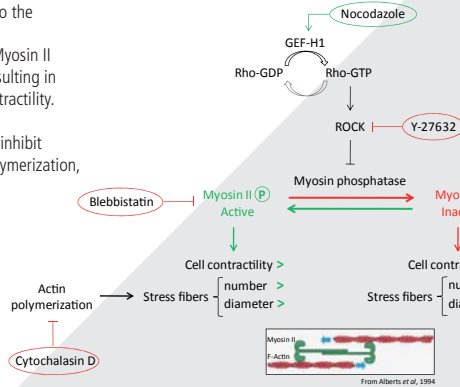
Adhesive Micropatterns: Cell shape and internal organization is the outcome of a complex interaction between the cell cytoskeleton and external conditions. When cells are plated on conventional culture dishes, the reproducibility of their architecture is completely lost due to a lack of spatial information. By controlling locations of adhesive (fibronectin, FN) and non adhesive areas (PEG), micropatterns result in a highly reproducible and polarized cell internal organization. Cells on L micropatterns adopt a triangular shape with acto-myosin contraction essentially on one edge giving rise to a single strong stress fiber.

Actin, nucleus, Fibronectin (FN),
PEG: poly ethylene glycol



Model summarizing the Rho-ROCK-Myosin signaling pathways that regulate cell contractility and the effect of drugs that activate (green) or inhibit (red) the pathway.

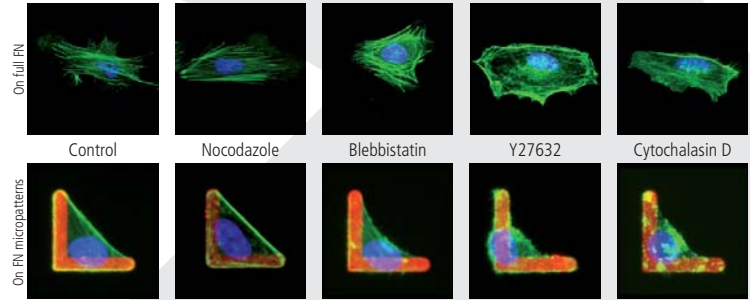
Nocodazole induces the release of GEF-H1 from microtubules into the cytoplasm increasing the level of Rho-GTP. ROCK and Myosin II are consequently activated resulting in an increase of stress fiber contractility. **Y27632, Blebbistatin and Cytochalasin D** respectively inhibit ROCK, Myosin II and actin polymerization, decreasing cell contractility.



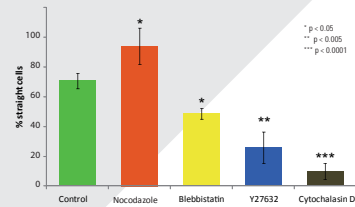
Unveiling drug-induced phenotypes on micropatterned cells

HeLa cells were seeded in parallel on full fibronectin or on fibronectin L micropatterns, then treated with drugs at 10µM for 1h (except for Nocodazole at 5µM) or left untreated.

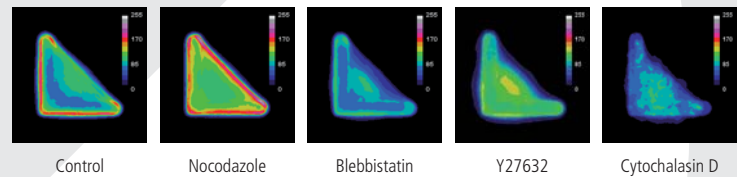
Nucleus; Actin; Fibronectin micropattern



Robust quantification of drug effects with only 50 cells



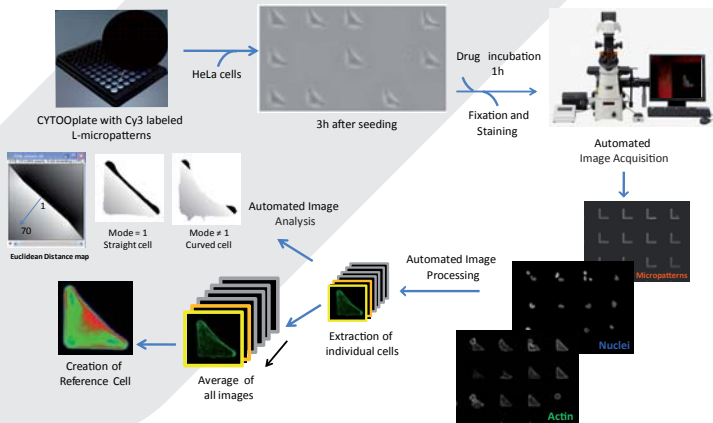
Actin Reference Cells



Cells seeded on L-micropatterns were treated for 1h with 4 drugs affecting directly or indirectly the actin cytoskeleton: Blebbistatin (10µM), Cytochalasin D (10µM), Y27632 (10µM) and Nocodazole (5µM).

The percentage of straight cells was determined using ImageJ macros as described in Methods. The graphs represent the average number of straight cells obtained from triplicates on a CYTOOplate. An ANOVA (analysis of variance) in conjunction with a Tukey's test were used to assess significant differences between control and treated conditions.

Methods

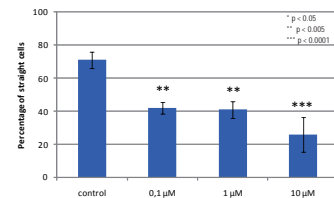


Automated Image Processing and Analysis were performed with dedicated ImageJ macros. For the analysis method, the Euclidean distance map (EDM) was used to distinguish between straight and curved cells. The EDM gives grey intensity values to each pixel as a function of its distance from the theoretical hypotenuse. Pixels immediately adjacent to the hypotenuse therefore have an intensity grey value of 1.

The shape of the cell is copied onto the EDM, then the mode (most frequent value of the distance map) is determined. Cells with a mode 1 correspond to straight cells while cells with a mode different from 1 are considered as curved cells. The percentage of straight cells is then calculated.

Reference Cells were obtained by averaging pixel intensity over a stack of aligned images.

A hundred-fold gain in sensitivity



Dose-response example for Y27632:

A significant effect of Y27632 can be measured at 0.1µM, a dose one hundred times less than what is usually used in cell based assays.

50 cells were analyzed from three independent wells. An ANOVA in conjunction with a Tukey's test were used to measure significant differences between control and treated conditions.

Conclusions

Taking advantage of the refined control of cell adhesion achieved on micropatterns, we present a method to quantify drug impact on cell contractility in a 96-well CYTOOplate format. Using L-micropatterns and 4 different drugs targeting directly or indirectly the actin cytoskeleton, we show that cell normalization can solve several bottlenecks in High Content Screening:

Acquisition and analysis: a regular array of micropatterns allows straightforward image acquisition, processing and analysis, all integrated into an automated workflow.

Sensitivity: opposing effects on cell contractility were efficiently detected and measured at low doses, while these effects are barely observable on regular cell culture supports.

Data collection: a significant difference between treated and untreated cells was detected with only 50 cells, while hundreds to thousands of cells are usually required for cell-based experiments.

Standard for comparison: The Reference Cell, a unique comparison tool created by averaging a stack of individual cells, clearly highlights visual intracellular differences between control and treated conditions.

