Build a Reference Cell™ for powerful cell phenotype quantification

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- Normalize cell positioning and architecture using adhesive micropatterns
- Co-map the distribution of an unlimited number of proteins and subcellular compartments
- Visualize and measure even subtle changes in cell response to RNAi or drug perturbations
- Quantify more variables using fewer cells
- Create your Reference Cell database

Background

Precise cell image quantification remains a real challenge in biology since cells seeded on a conventional Petri dish adopt different shapes and hence intracellular architectures. Here, we describe image quantification using innovative CYTOO micro patterns that allow cells to adopt the same shape and morphology. Such cell normalization results in a population of cells all exhibiting extremely reproducible internal architecture.

This Application Note describes the CYTOO approach to cell quantification and presents an easy method for generating a Reference Cell map, a new principle for observing and analyzing cellular changes. Using the Reference Cell procedure, cell phenotype quantification becomes extremely precise, sensitive and rapid.

Method

This experiment illustrates the methodology used to create a Reference Cell for actin.

1 . Normalize your labeled cells using CYTOO micropatterns

For this experiment, 2x2cm² CYTOOchips with FN550-labeled L-micropatterns were placed in a 6-well plate. 60,000 HeLa cells were seeded in each well, incubated for 15 min under the hood then moved to the cell incubator. After 30 minutes, cells started to spread. We immediately changed the cell medium and gently flushed the coverslip surface. All unattached cells were removed. The plate was then put back into the cell incubator for at least 3 hours to allow cells to achieve full spreading. Cells were fixed with PFA, permeabilized with Triton-X-100 and immunostained for actin using FITC-conjugated phalloidin. Nuclei were labeled with Hoechst.

2 . Acquire high resolution images

Fast automated image acquisition and display in this example used Metamorph imaging software. Automatic acquisitions were performed on a Nikon Eclipse Ti microscope equipped with a CCD Hamamatsu camera and an Intensilight mercury-fiber illuminator. Images were acquired at 20x magnification in three emission wavelengths (Fig. 1) corresponding to nuclei (blue), actin (green) and micropatterns (orange). The number of micropatterns visualized per field varies depending on the camera and objective setups. We thus created master stacks of images for all three channels.

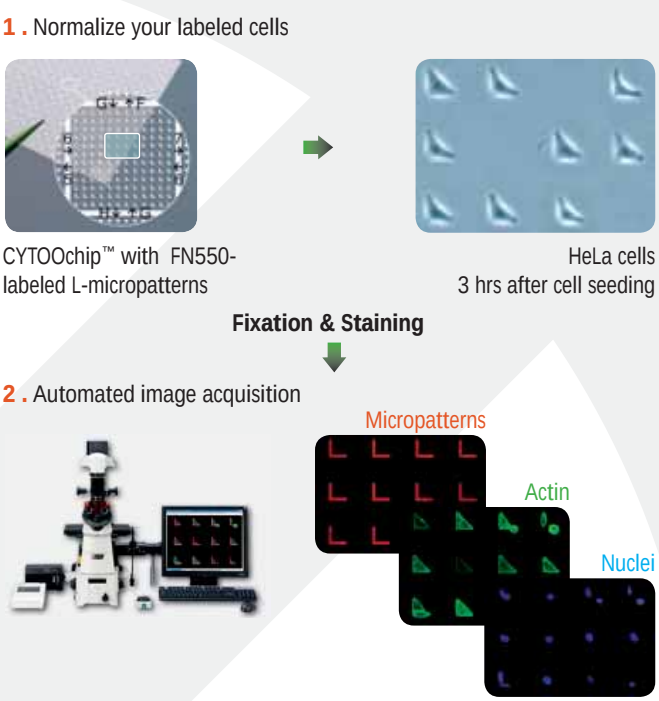

Figure 1. Overall CYTOO process for obtaining a Reference Cell (see text for details).

3 . Apply our Reference Cell ImageJ macro*** to isolate single micropatterned cells

Using the master micropattern stack, a sub-stack of individual micropatterns was created using the center of the micropattern to delineate and align the micropatterns. The position of each delineated region was recorded and transposed onto the master stacks of the other remaining channels. Individual micropattern images were cropped, extracted and reassembled into stacks.

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*** Request the latest version of the Reference Cell macro and a complete user's guide by emailing us at: contact@cytoo.com

AN001-V1.0 CYTOO 2011

To select for single cells, the macro detected the number of nuclei per image. If the nuclei count was different from 1, the slice was excluded from each of the stacks, generating final stacks for each of the three channels that now contained only single cells (an ImageJ realignment plug-in MultiStackReg can optionally be applied if images are not perfectly aligned). We thus generated final aligned stacks of individual images useful for single cell analysis or creation of a Reference Cell.

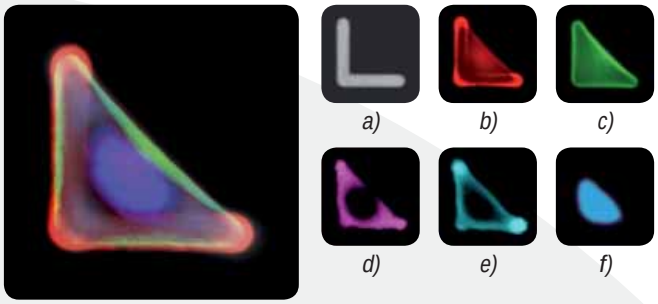

Figure 2: composite image (left) of multiple Reference Cells (right) a) Micropattern b) Paxilline, c) Actin d) Ezrin e) Cortactin, f) Nuclei.

4 . Calculate the Reference Cell

A Reference Cell was created by performing a Z-projection on each stack of filtered and aligned images. Among several methods available for Z-projection (e.g. mean, median, maximum intensity, etc), we applied the mean function. Finally, a Rainbow LUT (Lookup Table) was applied to the Z-projected image to facilitate examination and interpretation of experimental results.

The Reference Cell methodology represents the smartest way to get quantitative analysis of multiple targets of interest across many different treatment conditions. Indeed, you can bypass species cross-reaction between antibodies by applying numerous labels to the Reference Cell and without being limited by the number of fluorescent wavelengths available.

Application example: the Reference Cell for drug profiling

Figure 3 presents a gallery of Actin Reference Cells obtained after treatment of cells on L micropatterns with various drugs. Control cells on L micropatterns adopt a triangular shape with actomyosin contraction essentially on one edge giving rise to a single strong stress fiber. The Rho-kinase inhibitor Y-27632 and blebbistatin decrease cell contractility, while Nocodazole results in increased stress fiber formation. Cytochalasin D totally disrupts the actin network. Mapping actin after each treatment using the Reference Cell allows direct observation and comparison revealing subtle differences. Such an analysis allows you to create your own Reference Cell database to systematically map on a proteome-wide scale any given cell type and its organization in different functional states. Download the poster of this study here: www.cytoo.com/applications

Figure 3: Reference Cell gallery depicting drug effects on the actin distribution in cells on L micropatterns and performed in a 96-well CYTOOplate™. Nocodazole (5µM), Blebbistatin (10µM), Y27632 (10µM) and Cytochalasin D (10µM), n=50 cells for all conditions.

Conclusion

By using adhesive micropatterns, a detailed quantification of the Reference Cell allows precise and representative determination of features such as the actin cytoskeleton. This process leads to cell image quantification with outstanding sensitivity, repeatability and reliability.

We have illustrated the valuable and indispensable use of adhesive micropatterns to normalize cells and the easy steps required to elaborate a Reference Cell representative of the average cell population response. Our technology allows you to create your Reference Cell for any label of interest and build a Reference Cell database.

Related reading

Cell Motility and the Cytoskeleton, 63(6):341-55, 2006
Proc Natl Acad Sci U S A. 103(52):19771-6, 2006
Nature Cell Biology, 7(10):947-53, 2005
Watch our video on the JoVE website for a full visual guide to this Application Note:
JoVE 46: http://www.jove.com/index/Details.stp?ID=2514
www.cytoo.com/publications for more reading

Related product information

Cat. No	Product Name
10-014-12	CYTOOchips 20x20 L-M-FN550
20-014-12	CYTOOplates 96 L-M-FN550

The Reference Cell can be applied to all other standard (crossbow, H, Y...) or custom micropatterns.
Please visit www.cytoo.com/store to see available products.

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