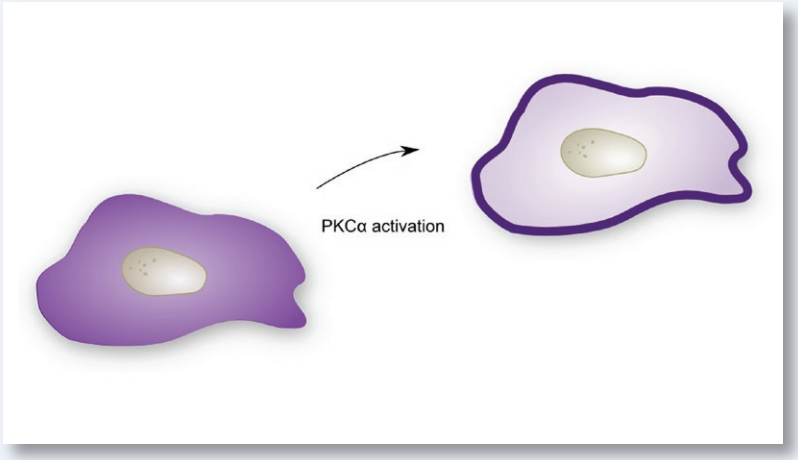

APPLICATION NOTE

Image-based Quantification of PKC Activation using Operetta

Key Features

- Reliable measurement of cytosol to membrane translocation
- Detection of PKC in plasma membranes using texture-based image analysis
- No membrane marker required

Texture Analysis of the Plasma Membrane

Background

Protein Kinase C (PKC) represents a family of serine / threonine kinases which help to regulate cell division and migration. These processes, when not controlled properly, can lead to tumor growth and metastasis [Newton, 1997, Nishizuka, 1984]. PKCα is the most relevant isoform of PKC for drug discovery. When activated, PKCα translocates from the cytoplasm to the plasma membrane where it interacts with regulators of cell cycle and motility pathways. Modulators of PKCα have shown potential as anti-tumor drugs [Konopatskaya and Poole, 2010].

PKCα translocation from cytosol to plasma membrane is an important High Content Screening application in cancer research. Previous attempts have been made to measure PKCα activation by quantifying the extent of its colocalization with a membrane marker or by measuring the accumulation of fluorescently tagged PKC in fluorescent spots. However, these types of evaluation of high content assays have proven to be unreliable and error-prone.

Here, we present a different approach to the analysis of plasma membrane bound PKCα signal which is independent of any membrane dye or spot accumulation. Our plasma membrane texture-based approach has been shown to be very robust, and accurately quantifies PKCα located in the ridge-like structures of membrane between neighboring cells.

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Application

We have developed an assay protocol to measure PKCα activation using an anti-PKCα antibody and a nuclear dye. HeLa (human cervix carcinoma) cells were seeded into a 384-well CellCarrier™ microtiter plate at a density of 10,000 cells per well and cultured overnight. PKCα was then activated by treatment with phorbol-12-myristate-13-acetate (PMA). After incubation for 10 minutes with various concentrations of PMA, the cells were fixed using 3.7% formaldehyde. The cells were permeabilized with 0.2% Triton X-100 and immunofluorescently stained using an anti-PKCα primary monoclonal antibody, followed by an AlexaFluor®555 secondary antibody conjugate (Cellomics® PKCα Activation HCS Reagent Kit, Thermo Scientific®). Nuclei were stained using 10 µM Hoechst 33342 dye solution. The plate was then imaged on the Operetta® High Content Screening system in widefield fluorescence mode using the 20X high NA objective. Five fields per well were imaged and analyzed (Figures 1-3).

The approach to image analysis in the Harmony® software was based on counting nuclei (Hoechst channel), followed by texture analysis (PKCα channel) using one of the 'SER Features' methods (Figure 1). This image analysis 'building block' analyzes the intensity structure of a defined image region for the occurrence of typical patterns. In this case the whole image, with the exception of the nuclear regions, was searched for typical 'ridge' shapes of 3 pixels in width. These 'ridges' represented PKCα associated with plasma membrane regions which could be quantified based on the frequency and intensity of the identified ridges.

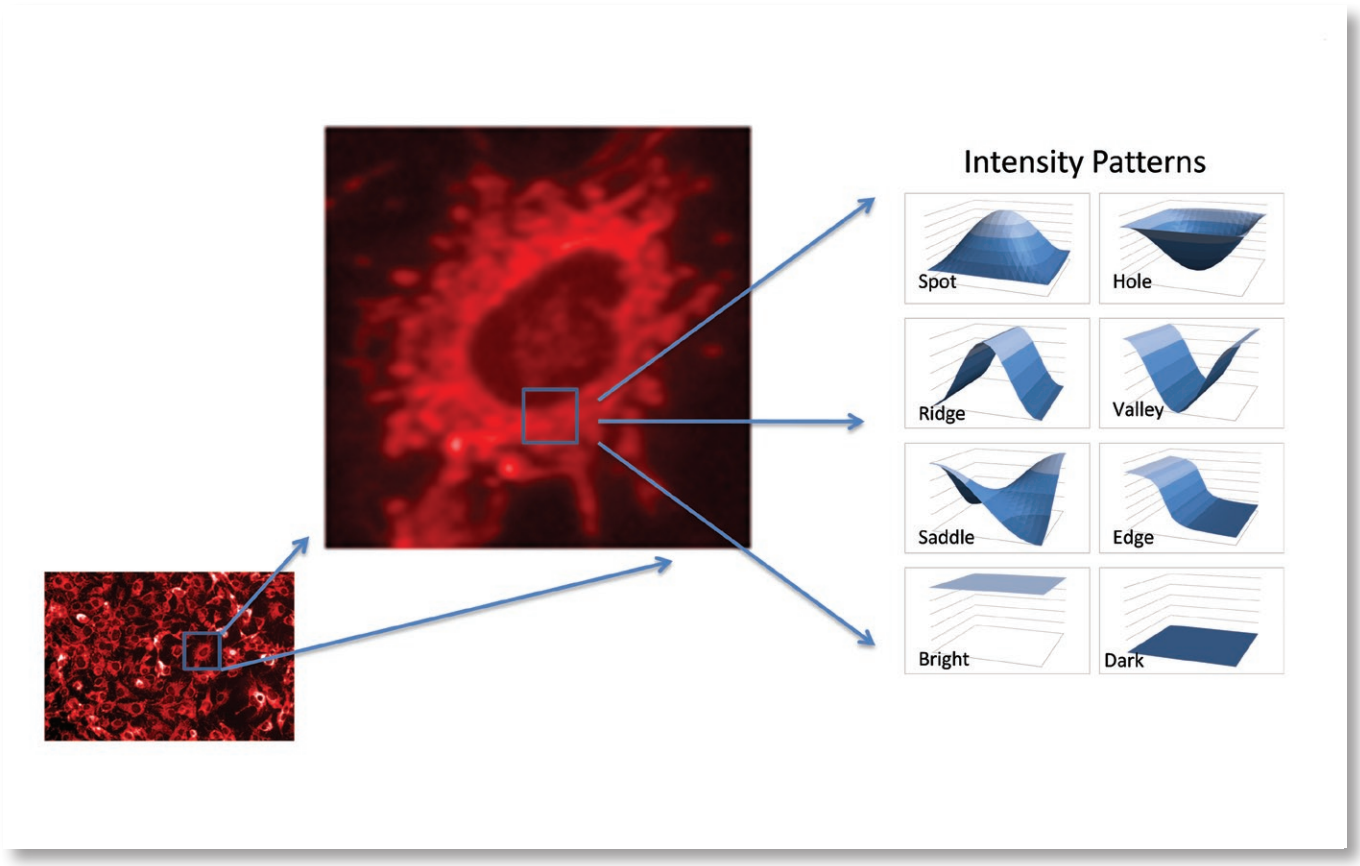

Figure 1. 'SER Features' (Saddles, Edges, Ridges) of the Harmony software. The intensity structure of a region (here: cytoplasmic region around a nucleus) is analyzed for the occurrence of typical intensity patterns, e. g. 'edges', 'ridges', 'spots'. Left: Cells stained with a single marker. Center: Enlarged detail of one cell from the image on the left. Right: Set of eight intensity patterns that can be searched for in the image region. The 'ridge' feature is best suited to fluorescence intensity accumulation in the plasma membrane region of neighboring cells.

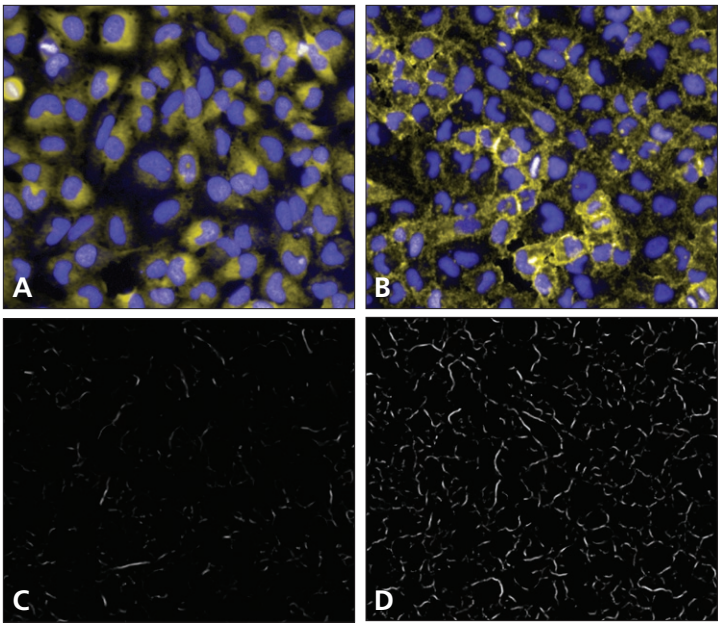

Figure 2. Texture-based image analysis for quantification of PMA-activated PKCα. Images A and B show false color overlays of nuclei (Hoechst, blue) and PKCα (AlexaFluor®555, yellow), A of non-treated cells and B of PMA treated cells. PKCα is localized in the cytoplasm and translocates to the plasma membrane upon compound activation. In image B, plasma membrane association of PKCα is clearly visible. Images C and D are the corresponding texture-rendered images of the PKCα channel in which membrane 'ridge' shapes are detected. The frequency and intensity of 'ridges' are increased in treated cells (D).

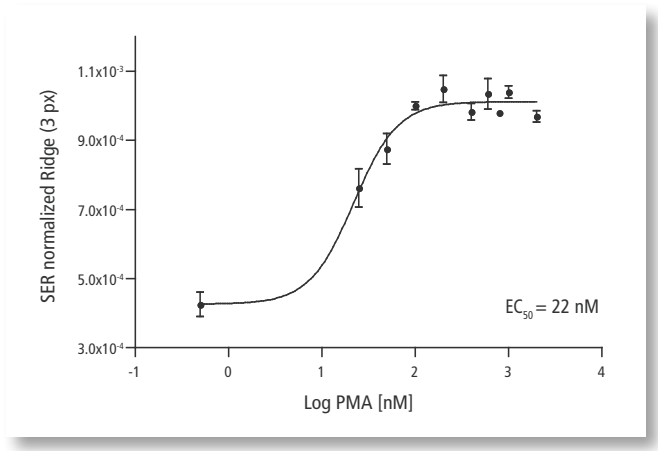

Figure 3. Dose dependent PKCα activation in HeLa cells stimulated with PMA. The SER 'ridge' signal, normalized by cell number and width parameter set to 3 pixels, increases as the PMA concentration increases. This signal represents PKCα association with cell membranes. N = 3 wells, z´= 0.55.

Conclusions

We have presented an easy and robust High Content Screening application for a cytoplasm to plasma membrane translocation assay using the Operetta / Harmony platform. Texture-based image analysis enables a direct identification of the labeled target molecule (PKCα) associated with plasma membrane regions and is therefore a direct and reliable quantification of PKCα activation. Using this approach, no additional membrane dye is required for the identification of plasma membrane regions, making the assay easier to set up and facilitating the use of further channels for other specific markers.

References

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