**Background**

Protein kinase C alpha (PKCα) is associated with a wide variety of cellular processes including proliferation, adhesion and motility. Over expression of PKCα appears to be associated with certain forms of cancer, and it has been identified as a biomarker for poor prognosis in breast cancer [Lønne *et al*., 2010]. Upon activation with phorbol esters, PKCα translocates from the cytosol to the plasma membrane [Chun *et al*., 1996]. For this reason, it is best studied with a high content analysis method that provides quantitative data on subcellular localization.

Tyramide Signal Amplification™ (TSA) is a widely referenced technology for providing greatly enhanced sensitivity in an extensive range of assays. It can be used in any application that allows the addition of horseradish peroxidase (HRP) to the protocol, such as *in situ* hybridization [Thompson *et al*., 2008], ELISA [Luk *et al*., 2009], microarray-based differential gene and protein expression studies [Karsten *et al*., 2002], immunohistochemistry [Zaidi *et al*., 2000] and immunofluorescence (IF) [Brouns *et al*., 2002].

Using TSA with a standard High Content Screening (HCS) assay for PKCα results in a significant increase in sensitivity without loss of resolution or increase in background. For these reasons, TSA technology is a useful tool in HCS, especially when detecting low-abundance targets in confocal mode. TSA allows the reduction of exposure time by an order of magnitude, resulting in a reduction of the time required for reading a plate.
Five fields per well were imaged and analyzed (Figures 1-3).

PKCα is localized in the cytoplasm and then translocates to the plasma membrane upon compound stimulation. We showed that TSA amplification did not influence the localization of PKCα signal (Figure 2), but reduced the exposure time by a factor of ten. Furthermore, TSA amplification allowed for 5X less primary antibody to be used compared to the standard protocol. Translocation of PKCα upon PMA treatment was clearly detectable both with and without amplification.

For quantification of PKCα activation, we used the texture image analysis module "SER Ridge" in the Harmony® image analysis software to identify typical membrane shaped “ridges” of 3 pixel width (Figure 3). The calculated signal, based on the frequency and intensity of identified ridges, represented PKCα association with cell membranes. The assay showed the same dynamic range with and without TSA amplification, however, the TSA results had a slightly increased signal to background ratio.
Figure 2. Comparison of PKCa signal without and with TSA amplification. Images show false color overlays of nuclei (Hoechst, blue) and PKCa (yellow). A, B | Non-amplified PKCa signal obtained using an exposure time of 8 s. C, D | TSA amplified PKCa signal obtained using an exposure time of 0.8 s using the 20X high NA objective in confocal mode. B, D | Cells were stimulated with 800 mM PMA. A, C | Cells were not stimulated.

Conclusions
We present here the integration of the Tyramide Signal Amplification (TSA) kit into a typical antibody-based high content imaging assay, activation of PKCa. The TSA enhanced signal of PKCa resulted in a significant increase in sensitivity, with an increased signal to background ratio and without loss of resolution. No adaptation of the image analysis strategy was necessary and cytosolic and plasma membrane signal detection allowed for reliable quantification of PKCa activation. Fluorescence signal amplification using TSA is a valuable tool for high content assays that suffer from weak fluorescence signals and require long exposure times.

Figure 3. Quantification of signal amplified PKCa activation in HeLa cells stimulated with PMA. The texture analysis module in the Harmony software was used to identify typical membrane shaped “ridges” of 3 pixel width.
References


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For further details on High Content Screening please visit www.perkinelmer.com/imaging
For further details on TSA please visit www.perkinelmer.com/tsa