APPLICATION NOTE

Following live cells through the cell cycle using Chromobodies imaged on Opera

Key Features

- Intracellular antibody
- Real-time visualization
- Identify S phase via texture analysis

Live cell, Cell cycle, Chromobody, Texture analysis

Background

Chromobodies® are a new class of fluorescent antibodies which are characterized by their extremely small size, high stability and excellent reproducibility. Most importantly, Chromobodies can be used in live cells to target and trace their endogenous antigens through different subcellular compartments, which is not possible with conventional antibodies [Rothbauer *et al.*, 2006]. Unlike classical GFP-fusion proteins, Chromobodies have the potential to detect and visualize non-protein components or specific post translational modifications of target proteins. These characteristics render them valuable tools as direct live cell biomarkers on High Content Screening platforms.

However, Chromobody signal quantification can be challenging. The fluorescence signal from bound Chromobodies must be identified against a background of unbound Chromobodies in the cell. Measuring the distribution of target molecules requires advanced image analysis tools.

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Application

Experiments were performed on HeLa cells stably expressing a cell cycle protein-specific Chromobody, fused to GFP. 4000 cells per well were seeded into a 384-well CellCarrier™ microplate in 50 µl DMEM growth medium and cultured overnight. The microplate was then transferred to an Opera® High Content Screening system equipped with an Environmental Control Unit (ECU). The atmosphere inside the Opera chamber was controlled at a temperature of 37 °C, 80 % humidity and 5 % CO_2. Images were acquired from a series of wells in parallel in 30 min intervals over a total period of 22 hrs using a 40X water immersion objective and 160 ms exposure time at low laser power.

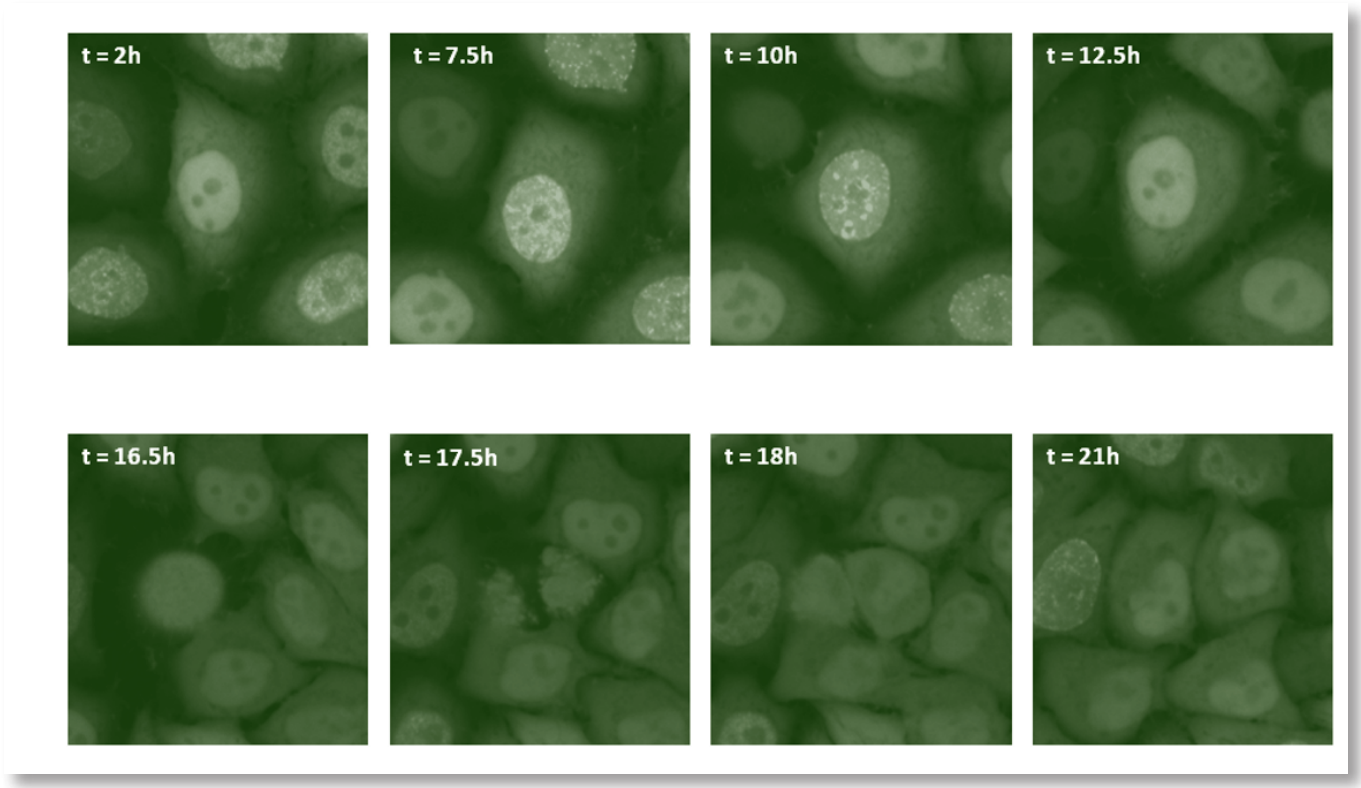

Figure 1. Chromobody signal during the cell cycle. Confocal images were captured using the 40X water immersion objective. To illustrate how the Chromobody binding signal changes over time, only a subsection of the acquired images are shown. The Chromobody signal starts as a homogeneous distribution through the nucleus and cytoplasm. Over time the nucleus begins to appear granular and forms spots, until finally the granularity disappears and the cell divides.

The fluorescence signal of the cell cycle protein-specific Chromobody used in this experiment is slightly enriched in the nucleus while showing a diffuse distribution throughout the nucleus and cytoplasm of cells. During S phase, the target protein is recruited to DNA replication sites. Upon binding of the Chromobody, the fluorescent signal in the nucleus becomes granular and bright spots appear. In G2 phase the granularity disappears again and the cells enter mitosis (Figure 2).

This transition, which reflects Chromobody binding, can be analyzed quickly and efficiently using the texture analysis features of the Acapella® image analysis software. Of the many texture analysis parameters that are available, the 'SER Saddle' (**S**pots – **E**dges – **R**idges, Saddle) parameter is best suited for quantifying the Chromobody signal, by measuring nuclear granularity. The increasing texture-based readout of the Chromobody signal reflected the S phase of the cell.

An additional readout was obtained using the cytoplasmic Chromobody signal to quantify the cytoplasmic area. By calculating this area, the M phase was easily identified by the sudden decrease in cell size after 16.5 hrs (Figure 3).

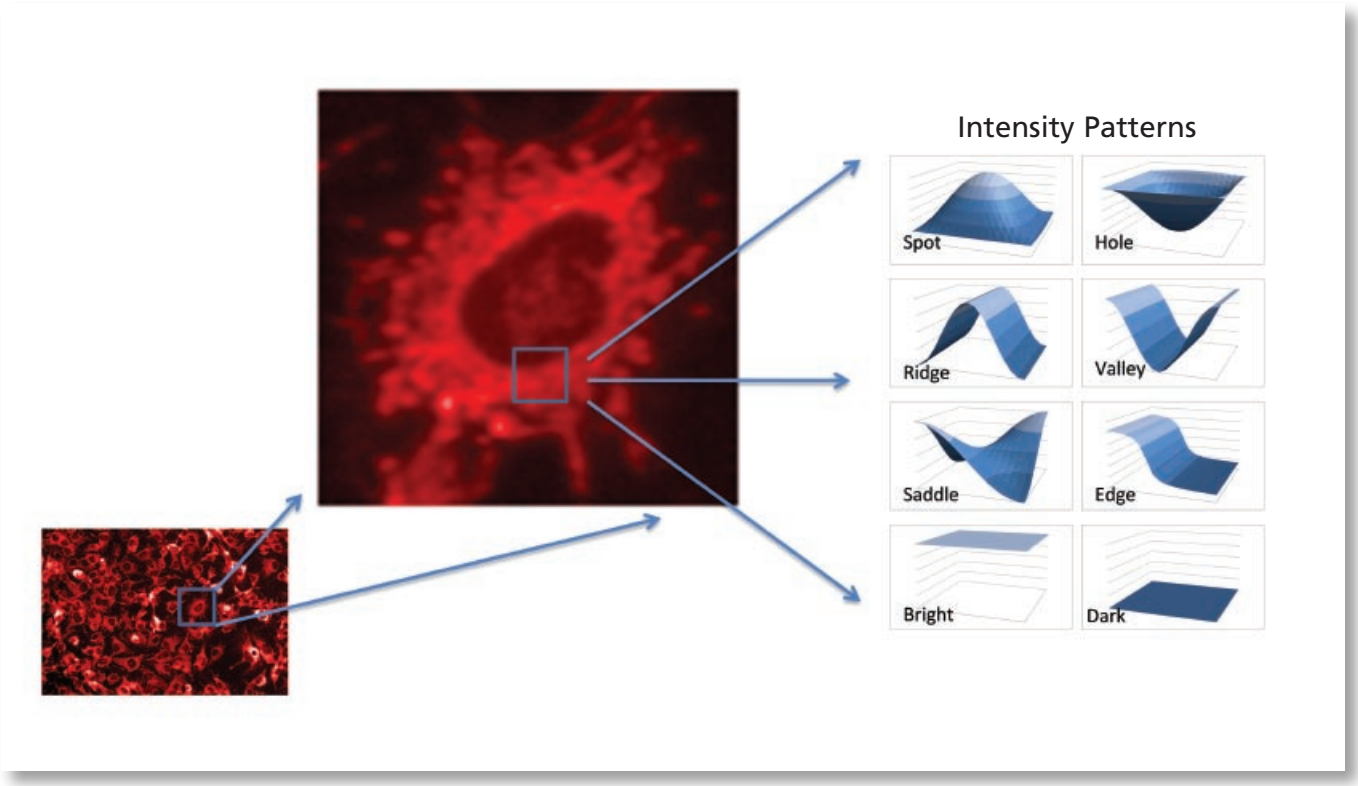

Figure 2. Texture analysis parameters from Acapella. The illustration shows the set of eight SER (Spots – Edges – Ridges, Saddle) texture analysis parameters. Analysis is carried out by selecting the parameter that provides the best results for measuring the change in Chromobody binding signal within the nucleus. In this experiment, the 'SER Saddle' parameter provided the best results by measuring nuclear granularity.

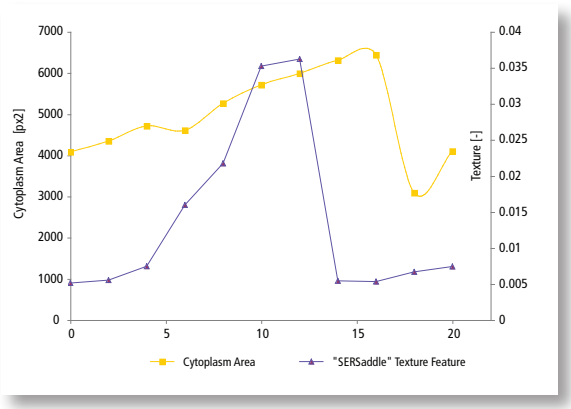

Figure 3. Following a single cell through a full cell cycle. The texture parameter ('SER Saddle') was used to measure the Chromobody cell cycle protein signal in the nucleus in order to identify cells in S phase (purple). Measurement of the cytoplasmic area was used to identify cells rounding up and entering mitosis (yellow).

Conclusions

We have demonstrated that fluorescently labeled Chromobodies can be used for cell cycle analysis in live cells using the Opera High Content Screening platform. We have shown that the S phase of the Chromobody-labeled cell can be determined using texture-based analysis, whereas the M phase can be detected based on measurements of the cell size. No further dye was needed for S phase and M phase identification, enabling the use of additional fluorescence channels for multiplexing with other high content imaging assays.

This method has great potential for determining the influence of compounds on the cell cycle of live cells in a High Content Screening scenario.

References

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009196_01 Printed in USA Jul. 2010