

1 Introduction

In this study we describe how single live cells can be followed through the entire cell cycle using a specific cell cycle protein binding cameloid antibody (Chromobody®) tagged with GFP (*green fluorescent protein*). Multiple samples could be analyzed in parallel on the Opera™ High Content Screening platform. It is equipped with environmental control and Nipkow spinning disc confocal fluorescence imaging technology low in photobleaching and phototoxicity particularly designed for imaging live cells over long periods of time.

2 Material & Methods

Experiments were performed in HeLa cells stably expressing the cell cycle protein specific Chromobody® fused to GFP.

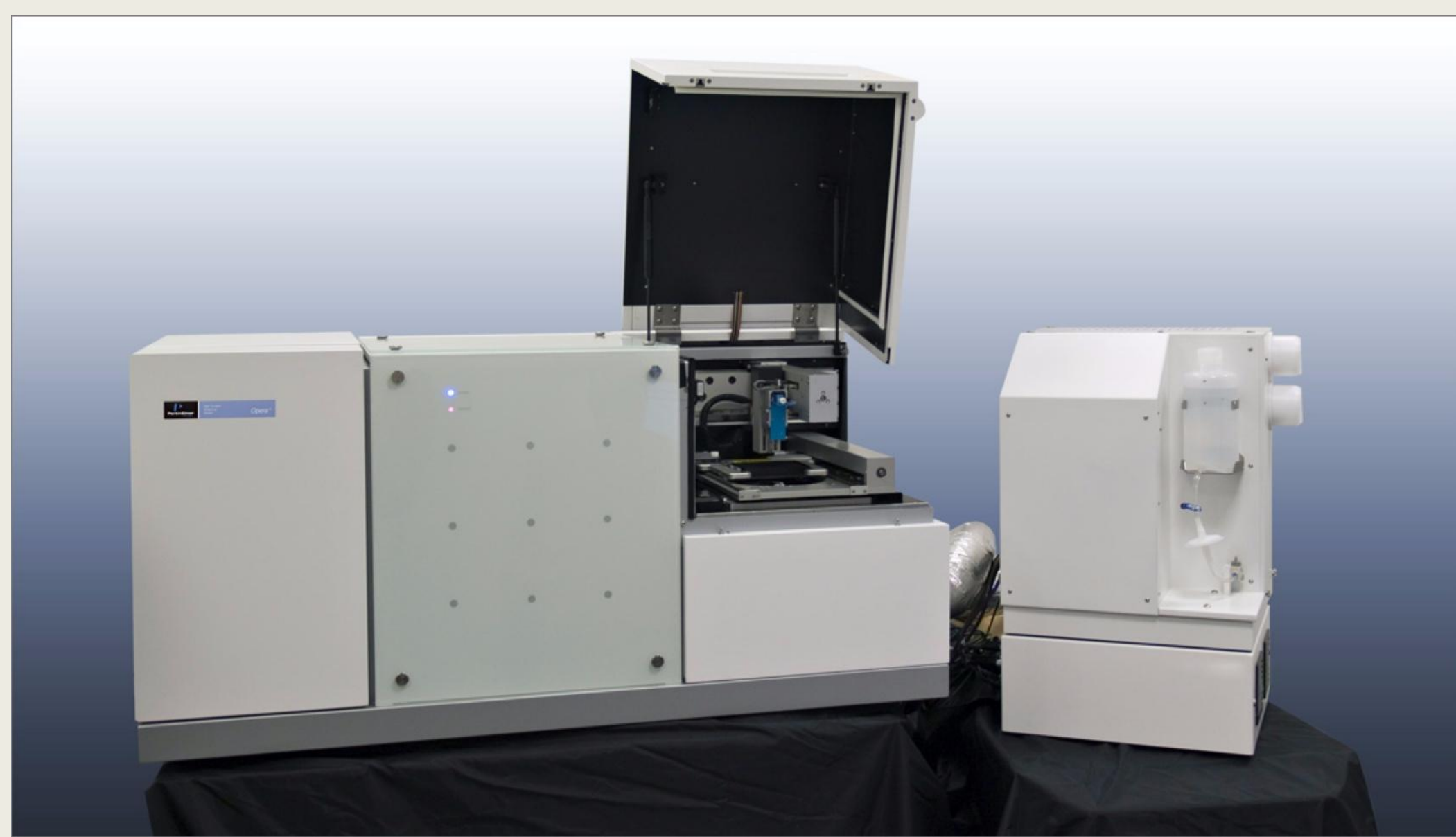


Fig. 1 | Opera™ platform with Environmental Control Unit (ECU)

4000 cells per well were seeded into a 384-well CellCarrier™ microplate in 50µl DMEM growth medium and cultured overnight. The microplate was then transferred to an Opera 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 % CO₂. Images were acquired from a series of wells in parallel in 30 minute intervals using a 40x water-immersion objective and 160 ms exposure time at low laser power over a total period of for 22 hours.

3 Chromobody® technology

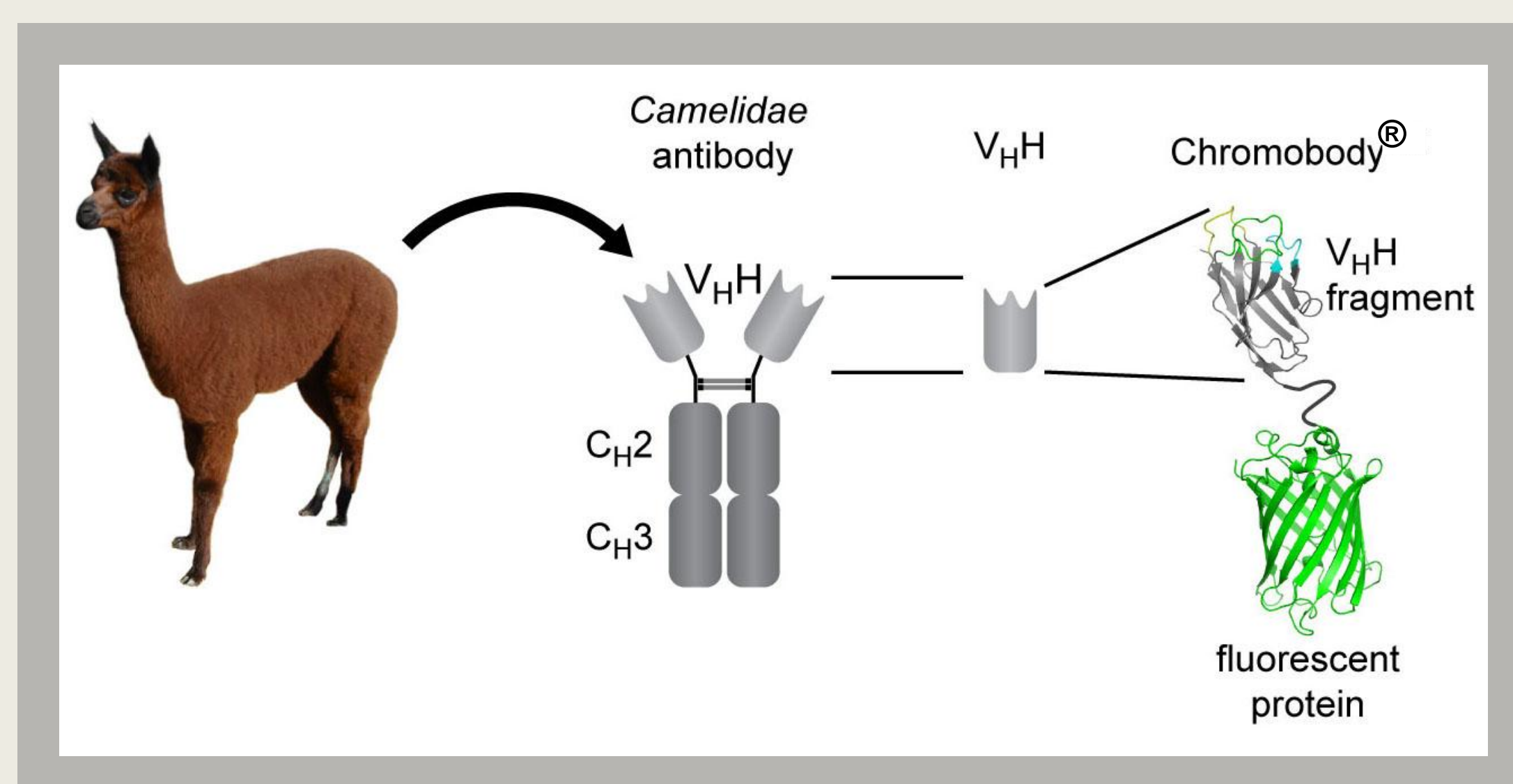


Fig. 2 | Schematic outline Camelidae-derived nanobody (V_HH) and its functional Chromobody® derivative.

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

In addition to cellular assays, Chromobodies® are ideal reagents for a multitude of analytical and preparative applications such as purification of fusion proteins, antigen detection or selection of transformants.

Key Features

- detection of endogenous proteins in living cells
- real-time visualization of posttranslational modifications
- no over-expression artifacts
- full flexibility of use in live cell or end-point assays

4 Cell cycle analysis using Acapella™ texture features on Chromobody® signal

The fluorescence signal of the cell cycle protein specific Chromobody® used in this experiment appears evenly distributed throughout the nucleus and cytoplasm of the cells, when not bound to its target. As soon as the target protein is expressed the fluorescence of the nucleus gets granular followed by the appearance of brightly fluorescent spots. The granularity disappears again and finally the cell divides (Fig. 3).

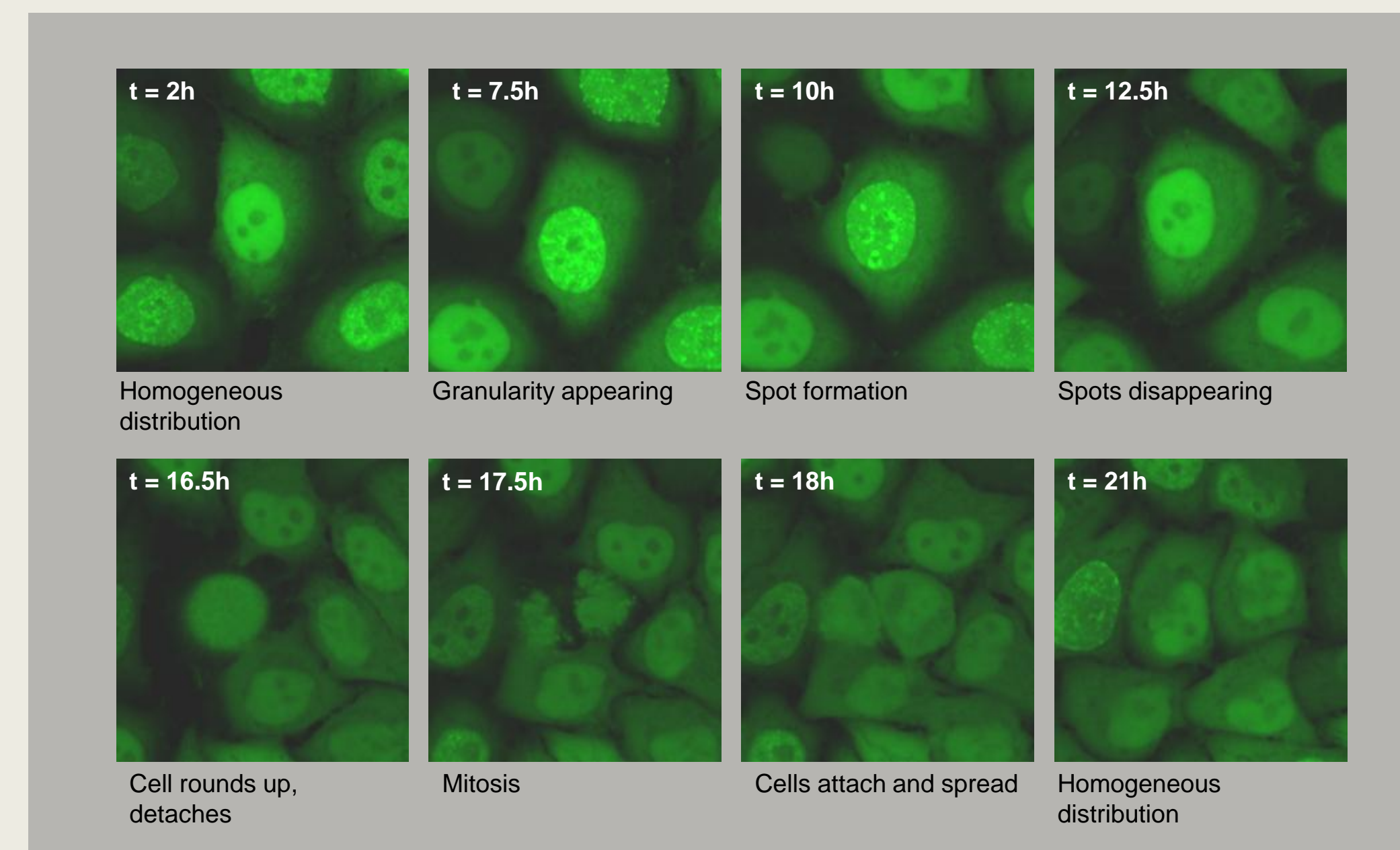


Fig. 3 | Chromobody® signal during cell cycle. Images show a subsection of Opera images, 40x water immersion objective. A HeLa cell is followed through a full cell cycle.

This transition which reflects Chromobody® binding can be analyzed very fast and efficiently using the new texture analysis features of Acapella. Of all the texture parameters available the "SER Saddle" (Spots - Edges - Ridges, Saddle) parameter is best suited for the Chromobody™ signal.

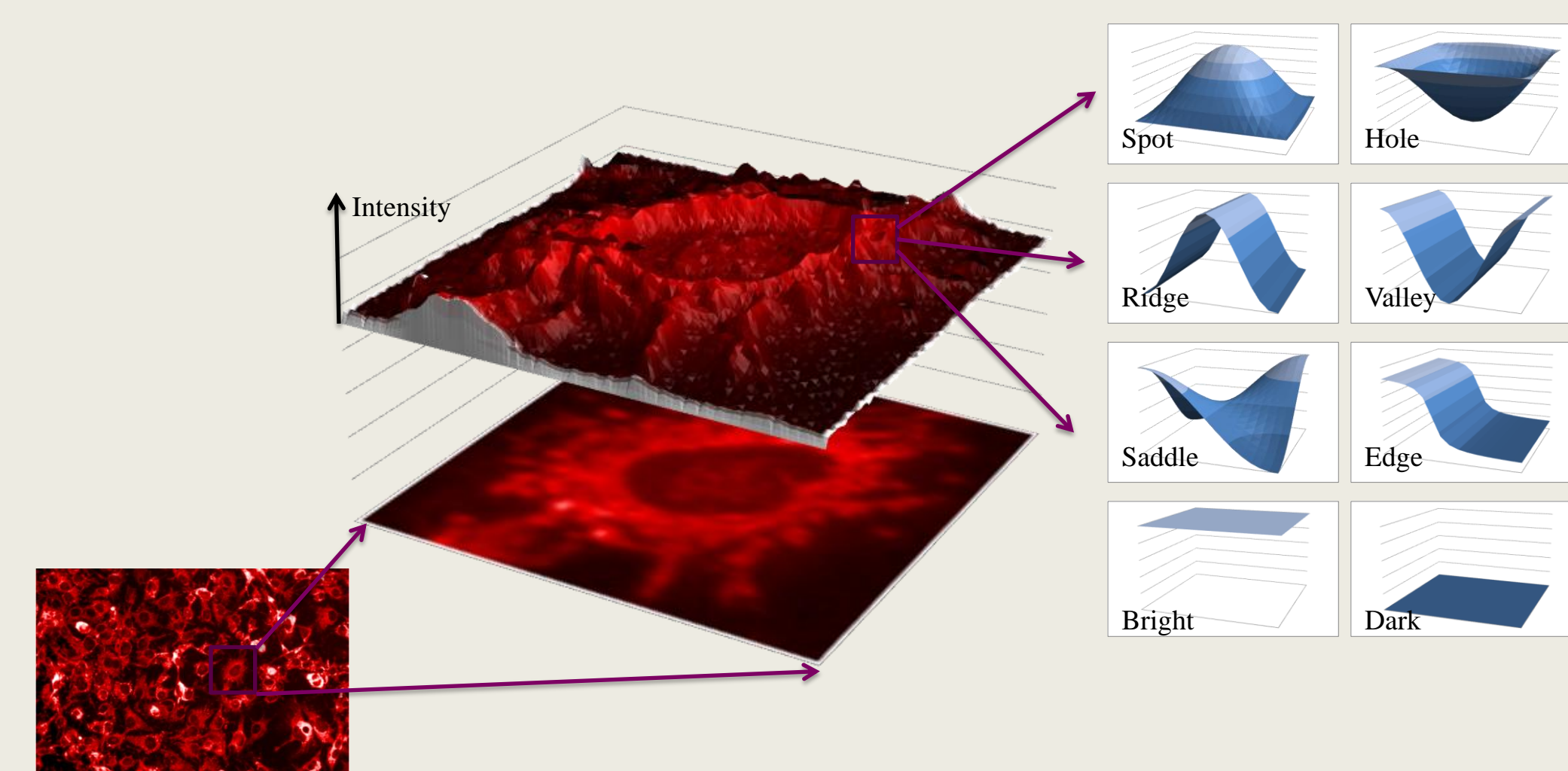


Fig. 4 | Texture analysis parameters of Acapella. The schematic on the right shows the set of eight SER texture analysis parameters. Analysis is done by selecting the parameter which provides best results for measuring the change of intensity distribution in a particular area of the cell. In our case it was the "SER Saddle" (Spots - Edges - Ridges, Saddle) parameter for chromobody distribution in the nuclei.

Cells and cytoplasm area were identified based on nuclear and cytoplasmic Chromobody® signal. The texture of the nuclear Chromobody® signal and the area of cells were used as readout.

One individual cell was selected manually for visualizing its transition through cell cycle and identifying S and M-phase based on nuclear texture and cell area (Fig. 5)

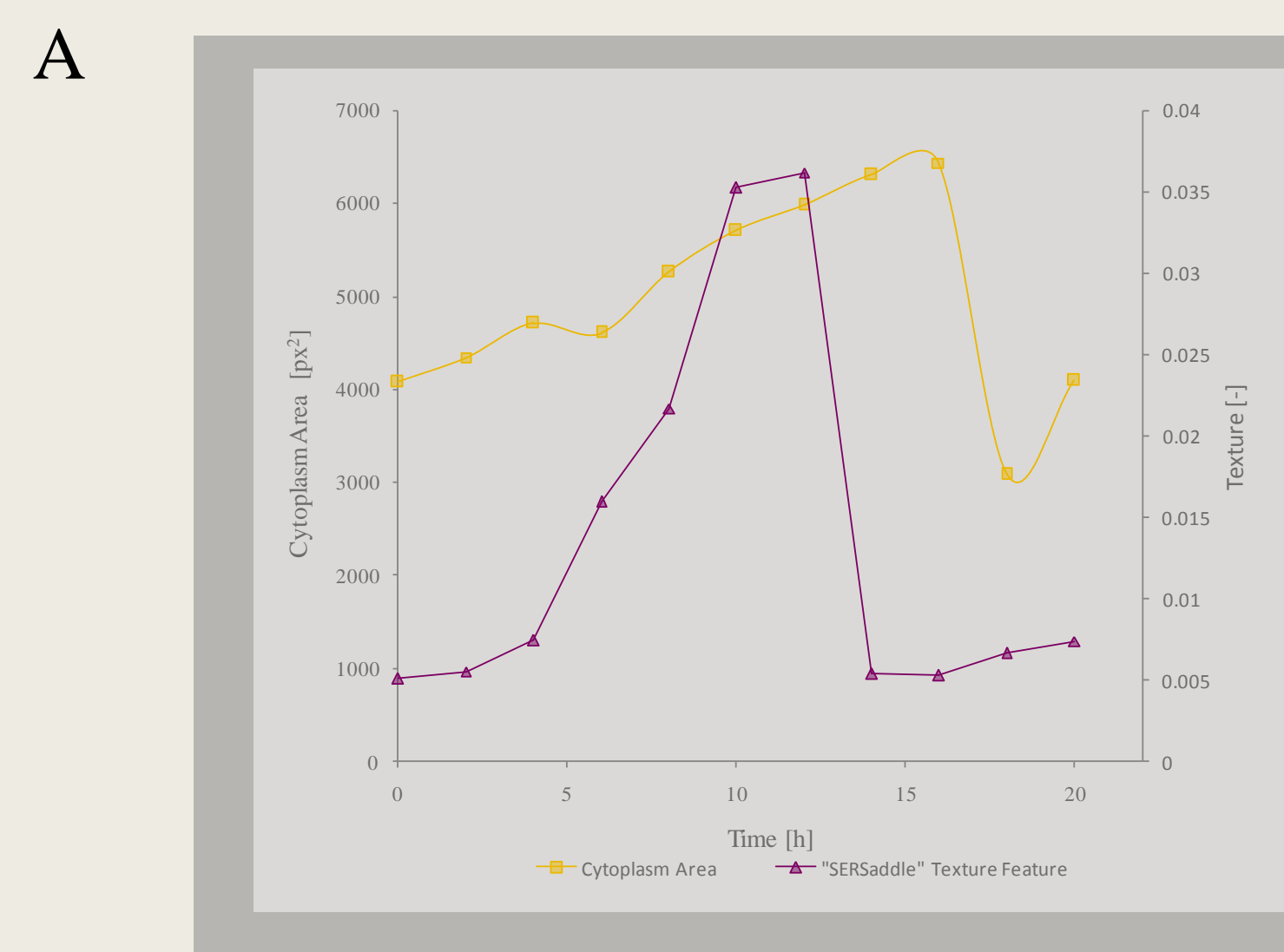
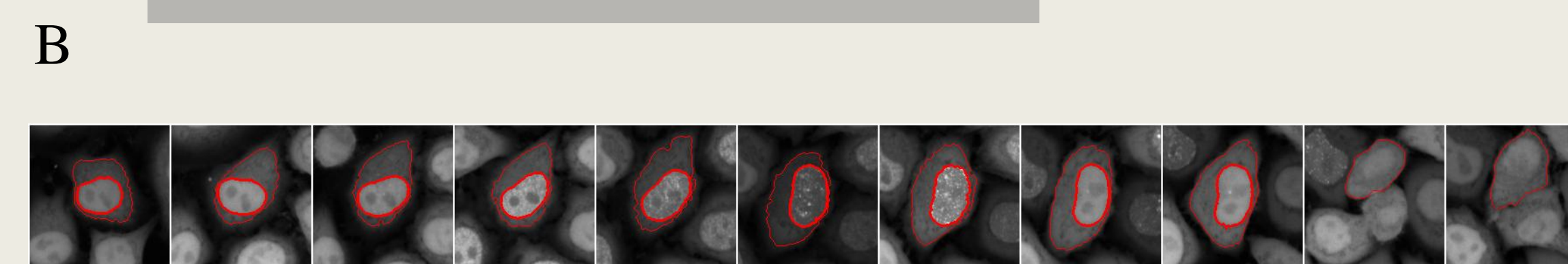


Fig. 5 | A - Following a single cell through a full cell cycle. The texture parameter („SER Saddle“) was used for the Chromobody® - cell cycle protein signal in the nucleus to identify cells in S-phase (purple). Cytoplasm area was used to identify cells rounding up and going into mitosis (yellow). B - Exemplary segmentation of one cell through the cell cycle.



5 Summary

We could show that fluorescently labeled Chromobodies® can be used for cell cycle analysis in live cells employing the Opera High Content Screening platform.

Cells in S and M phase can be distinguished based on Chromobody® signal and cell size, a nuclear dye can be added for distinguishing G₁ and G₂ based on DNA content. This method has potential for identifying the influence of compounds on the cell cycle of live cells in a High Content Screening scenario.