Automated Rapid and Accurate Cell Cycle Analysis with High-Content Imaging

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Abstract

Analysis of the cell cycle progression provides essential information in screening campaigns during drug discovery and development. The discovery of Chromobodies® and advancements in high-content imaging systems with improved optical properties has opened the door for new reliable and robust screening assays that provide relevant high quality data. Chromobodies are a novel class of fluorescent antibodies, based on special heavy chain antibodies from camelids (VHHs). These biosensors enable the non-invasive imaging of endogenous proteins and processes in real time. Therefore they avoid any deleterious side effects from the traditional over-expression of a tagged protein. This is especially true for PCNA whose high over-expression can have adverse effects on normal progression through S- phase. In this study we present a flexible and robust high content screening assay based on this technology in conjunction with high-content automated imaging and imaging cytometry platforms. We show dose-dependent cell cycle inhibition caused by a number of anti-cancer drugs including mitomycin C, paclitaxel, etoposide, doxorubicin, and cytarabine. Multiple analysis protocols enable the fast and accurate analysis with quantitative information on each sub phase of DNA replication for each individual cell as well as apoptosis, and allows to define IC50s for cytostatic and cytotoxic effects of compounds

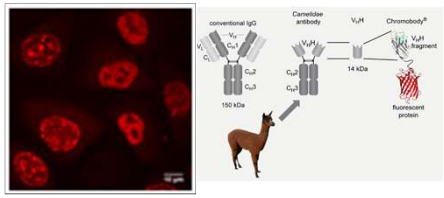


Figure 1. Schematic of process to create Chomobodies from camelidae antibodies.

ChromoTek's Chromobodies® are small intracellular functional antibodies based on the antigen binding domain (VHH) derived from heavy chain antibodies of camelids genetically fused to a fluorescent protein e.g.TagGFP or TagRFP suitable for real time analyses and perfect reagents for High-Content Analysis (HCA).

Materials & Methods

Chromobodies and Stable Cell Lines

- HeLa cells stably transfected with CCC-TagRFP were received frozen from ChromoTek. Cells were thawed and plated according to ChromoTek protocol.
- Cells were plated 4 K/96well plate or 1 K/384 well on standard Costar or BD plates andand treated with compounds next day.
- Data were collected at different time points, 8h-72h

High Content Imaging

- Image acquisition was done on the ImageXpress™ Micro XL System using 20x or 10x objectives with TRITC excitation and emission filters.
- One image per well was acquired using an exposure of ~200 msec
- Image Analysis: Images were processed in MetaXpress™ Software using either the Granularity Module or Cell Scoring Module.

Cell Cycle Indicator

A novel chromobody constructs have been developed for cell cycle analysis. The probe has been shown to detect antigens in chromatin and replication complexes and allow visualization of dynamic changes during the cell cycle in real time The ability to monitor replication loci is particularly useful for high-content imaging where complex image analysis techniques can provide multi-parametric results with a single color stain.

Representative images of a stable HeLa cell line is shown in Figure 2.

- Non-dotted nuclei are either G1 or G2.
- The signal intensity increases over time, therefore G2 usually is a bit brighter than G1.
- High intensity non-dotted nuclei are very early in S phase. At the very beginning of S phase, the nuclear signal suddenly increases, since PCNA is enriched in the nucleus, but the replication foci (at that time point very many, tiny spots) are not yet clearly visible and it appears like non-dotted.

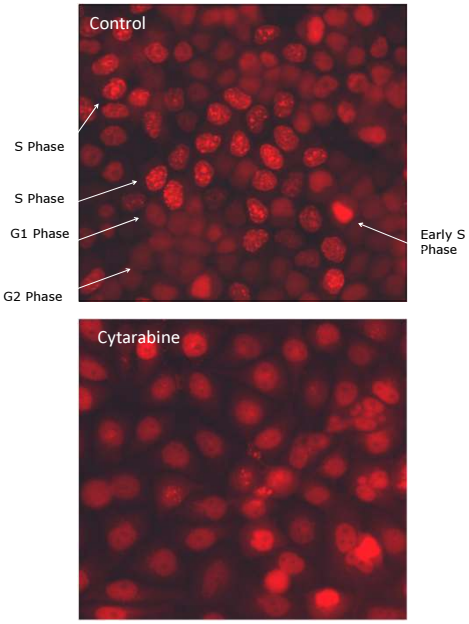


Figure 2. HeLa cells expressing CCC chromobody with a TagRFP. **Top:** Untreated (control) well. **Bottom:** Cells treated with cytarabine.

Granularity & Nuclear Characterization

A standard image analysis methodology is to identify a cellular region and then look for punctation or aggregation within that region. Often this can require two-colors (e.g., nuclear stain + punctate stain) which leads to longer acquisition and analysis times. The CCC probe alleviates the need for a second color as the TagRFP provides sufficient signal to segment cells, and aggregation during the different cell cycle phases is distinct enough to allow robust image analysis. Typical results are shown below in Figure 3.

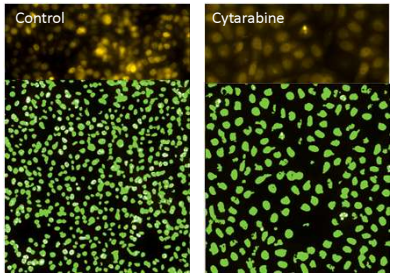


Figure 4. HeLa cells expressing CCC analyzed with Granularity module. **Top:** Representative images of Untreated (control) well and cells treated with cytarabine. **Bottom:**. Analysis results. White dots are cells in S phase. Green- nuclei.

Cell Scoring Analysis

A second image analysis method, Cell Scoring, was evaluated that provides faster time-to-results. In this case cells are segmented and then seperated into different populations based on area and intensity (i.e. brightness). Good distinction between G and S Phases was found with this method. Representative images are shown in Figure 4.

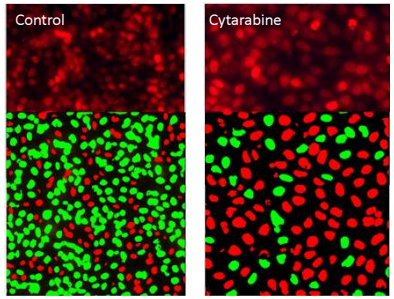
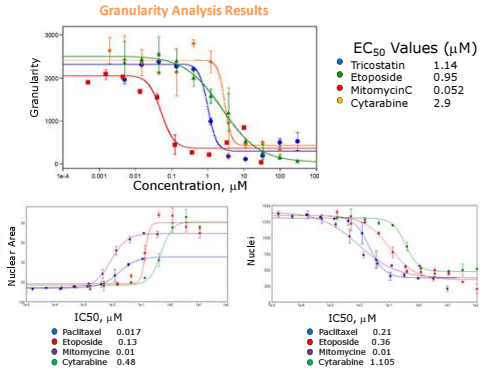


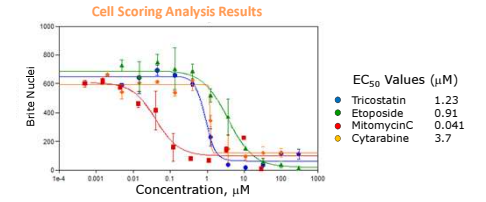
Figure 5. HeLa cells expressing CCC analyzed with Cell Scoring module. **Top:** Representative images of Untreated (control) well and cells treated with cytarabine. **Bottom:**. Analysis results. Green cells are in S phase while Red cells are arrested in G phase.

Anti-Cancer Drug Responses

The assay was tested with four known anti-cancer drugs: Etoposide, Cytarabine, Trichostatin A, and Mitomycin C. Concentration response was measured in 384 well plates using 2:1 serial dilutions. Analysis was performed with both Granularity and Cell Scoring modules. Results, presented below showed expected results including:

- Decreased cell number with replicating DNA (S-phase)
- Increased average nuclear size
- Decreased cell number





Summary

- We developed assay techniques using Cell Cycle Chromobody for measuring the impact of pharmacological compounds on cell cycle. The single-color nature of the method allows the possibility to combine this assay with other biological readouts.
- Two analysis modules were evaluated: Granularity for more detailed break-down of cell cycle phases, and Cell Scoring for efficient division of cells into G and S phases.
- These methods are well suited for High-Content Imaging environments with robust signal, reproducible result, and expected rank-order concentration responses of known anti-cancer agents.

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