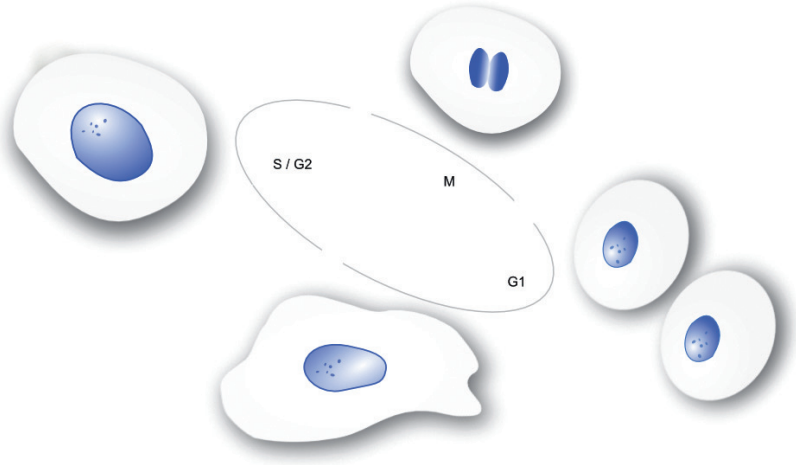


APPLICATION NOTE

# Image-based DNA Content Analysis during Cell Cycle using the Opera

**Key Features**

- Confocal image acquisition of fixed cells using the Opera™ High Content Screening system
- Analysis of DNA content distribution
- Image analysis using the Acapella™ DNA Content Distribution (DCD) Script

## DNA content distribution

### Background

The cell cycle consists of four distinct phases: G1-phase, S-phase, G2-phase (collectively known as interphase) and M-phase. During M-phase two tightly coupled processes occur: mitosis, in which the cell's chromosomes are divided and distributed to two new daughter cells, and cytokinesis, in which the cell's cytoplasm divides forming two distinct cells. One of the most important tasks in anti-cancer treatment is the inhibition of cell proliferation by interruption of the cell cycle. The different stages of cell cycle can be discriminated by determining the amount of DNA in each cell using a DNA stain. DNA synthesis during S-phase leads to an increase in DNA stain intercalation with an accompanying increase in fluorescence intensity. In G2/M-phase the cells will fluoresce with twice the intensity as in G1-phase. During the S-phase, the amount of DNA constantly increases, due to DNA-replication.

### Application

The DNA content of HeLa (human cervix carcinoma) cells treated with Nocodazole, Aphidicolin and Demecolcin during the different cell cycle phases was quantified using the nuclear dye DRAQ5™ and the Opera High Content Screening system. Nocodazole is an antineoplastic agent which exerts its effect by depolymerizing microtubules (arrest in G2/M-phase). Aphidicolin inhibits the growth of eukaryotic cells by selectively inhibiting the cellular replication of DNA polymerase II (arrest late G1/S-phase) and Demecolcin is a phytogenic antineoplastic agent (arrest in M-phase).

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DNA Content Distribution (DCD) histograms were created using the Acapella™ 2.0 DCD Script. The Script is based on the CellRegionAnalysis Script adding a configurable DNA content histogram creation step. The analysis assumes that nuclear DRAQ5™ fluorescence intensity signal is proportional to DNA content in the nucleus.

The histograms showed two clearly separated peaks, a G1 peak and G2/M peak (Figure 2). Untreated nonsynchronized HeLa cell cultures showed a typically shaped histogram containing two peaks and a plateau (S-phase) in between (Figure 2-I). The left-side major peak represents G1/G0 cells with a single chromosome set (2N), the right-side minor peak represents G2/M cells containing a double chromosome set (4N). Very late M-phase cells (Telophase, Cytokinesis) appear left of the G1 peak and not right of the G2/M peak, because they are already recognized as single cells.

The effects of the compounds Nocodazole, Aphidicolin and Demecolcin on the DCD histograms were as expected. Aphidicolin shows an increase of cell number in G1 and decrease of cells in G2/S (Figure 2-II). Demecolcin increased the G2/S peak as well as the S-phase plateau (Figure 2-III). Nocodazole also increased cell number in G2/M-phase (Figure 2-IV).

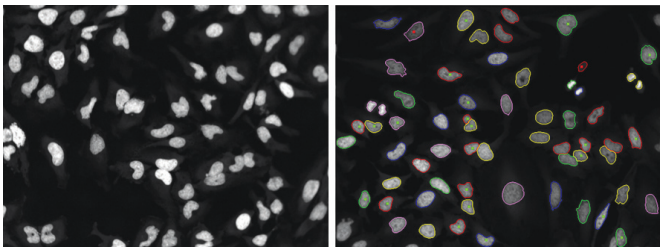

*Figure 1:* Opera images.
Confocal images of HeLa cells stained with DRAQ5™ using the 20X water immersion objective *(left)*.
Image analysis strategy for quantifying DNA intensity in HeLa cells with the Acapella script DCD. Red-spots: Nuclei with a mean DNA intensity < 1000; Green-spots: Nuclei with mean DNA intensity >1500 and < 2000 *(right)*.

## Conclusions

In summary, this method allows the analysis of cell cycle phases based on DNA content even with as little as 2000 cells. In addition, it can easily be multiplexed with many other biomarkers, such as Cyclins, p-Histones, BrdU and Rb.

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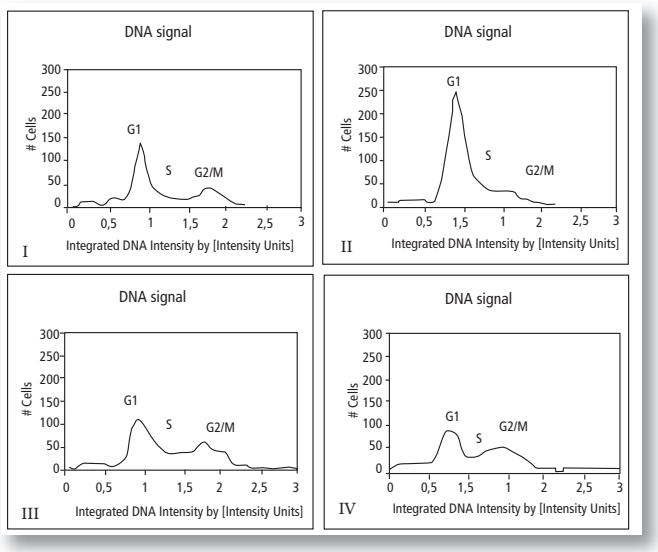


*Figure 2:* DCD subpopulation histograms showing cells distributed in G1/S and G2/M peaks. Subpopulations are differentiated by the integrated intensity of the DRAQ5™ stained DNA fluorescence.
I. Normal distribution of cells without treatment.
II. Distribution after 6 h of treatment with Aphidicolin (500 ng/ml); the fraction of cells located in the G2/M-phase has clearly decreased compared to the non-treated cells (figure 1, left), cells accumulate in G1- and early S-phase.
III. Distribution after 6 h of treatment with Demecolcin (500 ng/ml); the number of cells forming the G2/M peak as well as the S-phase plateau has increased.
IV. Distribution of cells after treatment with 500ng/ml of Nocodazol for 6 h. The plateau as well as the second peak are similar to III but with its maximum shifted more towards the S-phase which therefore appears smaller.

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008182A_06 Jan. 2010