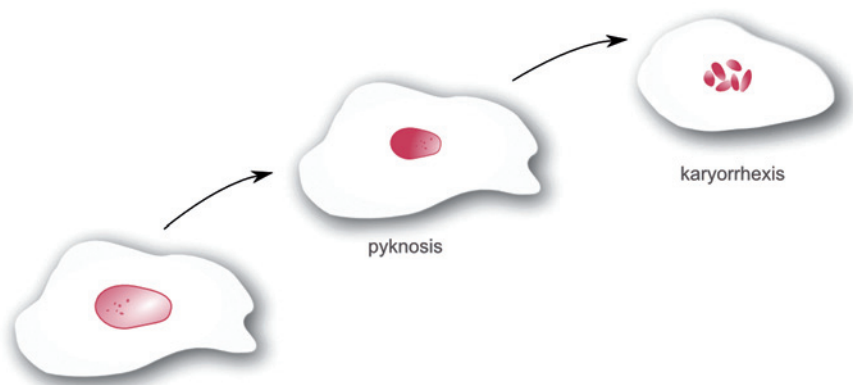


## Image-based Quantification of Apoptosis-Driven Nuclear Fragmentation using the Opera



### Key Features

- Confocal image acquisition of fixed cells using the Opera™ High Content Screening system
- Image-based quantification of characteristic changes in the nuclear morphology
- Acapella™ analysis using the Nuclear Fragmentation Script

## Karyorrhexis, pyknosis

### Background

Apoptosis or programmed cell death is the most common form of eukaryotic cell death and normally occurs during development, aging and as a homeostatic mechanism to maintain cell populations in tissues [Kerr 1972]. Cells undergoing apoptosis display profound structural changes, including a rapid blebbing of the plasma membrane and nuclear disintegration.

In this High Content Screening application we focus on the quantification of characteristic changes in nuclear morphology during apoptosis in a cell population which is triggered with an apoptosis inducer. Healthy cells possess the largest and most evenly stained nuclei, while early apoptotic nuclei appear condensed (pyknosis) and later on increasingly fragmented (karyorrhexis). The term nuclear fragmentation in this context describes the segmentation of the nucleus into smaller, compact compartments.

By combining this morphology-based method with other imaging read-outs, apoptosis can be approached on a multiparameter level leading to a reliable, validated high content assay.

## Application

In this report, apoptosis was induced in HeLa (human cervix carcinoma) cells with staurosporine, a broad-spectrum kinase inhibitor known to activate the apoptosis pathway. To generate dose-response curves, the drug was serially diluted (0 - 30  $\mu\text{M}$ ) and was added to the cells for 4 h. After incubation cells were fixed (3.7% formaldehyde) and stained with 10  $\mu\text{M}$  DRAQS™. The resulting images were acquired confocally on the Opera High Content Screening system with a high NA 20X water immersion objective.

Acapella image analysis was performed by applying the NuclearFragmentation Script. This script describes the apoptotic state of a cell population by evaluating changes in cell number as well as nuclear size, mean intensity and the degree of nuclear fragmentation. The scripting strategy used for the task is based on combining a compartmental segmentation step with a regional border adjustment of the search mask. Within the mask a texture analysis is performed and a statistical output is chosen to be the nuclear fragmentation index (Figure 1).

The assigned indices are directly related to the fluorescence intensity fluctuation of the nucleus stain; in fact, they are the CV value thereof (coefficient of variation, standard deviation divided by mean value). Figure 1 shows that the nuclei of healthy cells appear not only larger but more evenly stained compared to the apoptosis-driven fragmented nuclei. The distribution of the dye is more spotty in appearance. This effect can be used as an indirect indicator for the degree of the nuclear fragmentation.

By applying this image analysis method to the experimental setup an increasing degree of visible nuclear fragmentation can be translated into a dose-response curve, as shown in Figure 2B.

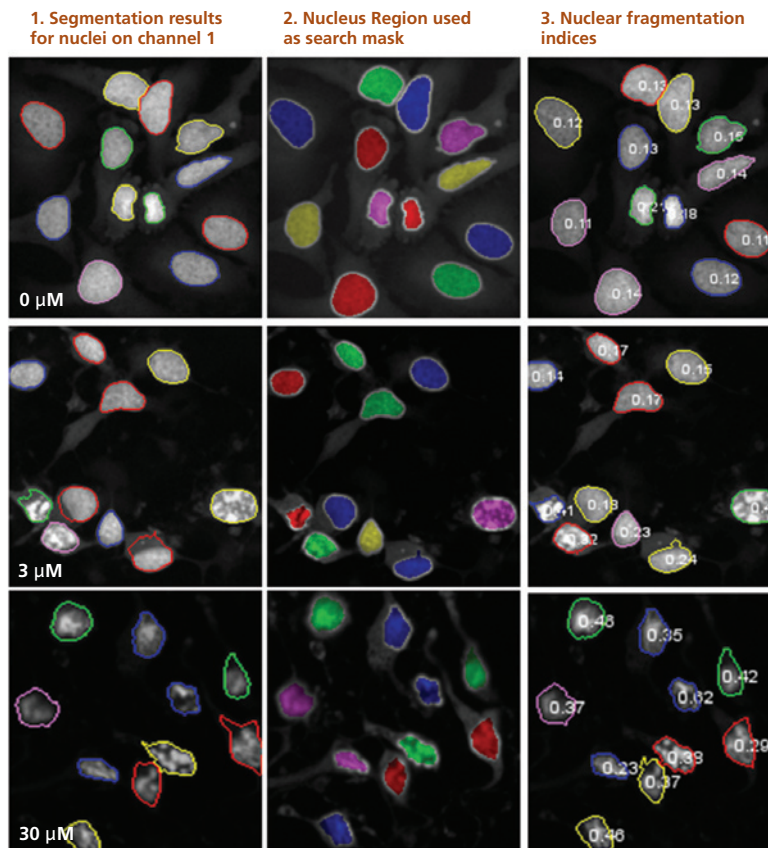


Figure 1: Image analysis strategy for indirectly quantifying nuclear fragmentation in HeLa cells after a 4h treatment with 0, 3 and 30  $\mu\text{M}$  staurosporine with the NuclearFragmentation. Evaluation is based on the fluctuation of the nuclear intensity.

Panel 1: segmentation results after applying the nuclei detection algorithm, DRAQS™-stained nuclei are detected on channel 1.

Panel 2: nucleus region created by diminishing the nucleus mask from channel 1, excluding the outer 2 border pixels minimizes.

Panel 3: numerical values of indices increase with increasing degree of the nuclear fragmentation.

## Conclusions

The apoptotic switch of the cell population can be validated by a well recognized morphology-based apoptosis marker – the degree of nuclear fragmentation. This marker can be easily combined with additional read-outs to confirm the effects of compounds on the apoptotic pathway in a cell population. It is simple and elegant as it only requires a nuclear stain which is present in almost any high content application for the segmentation of the image.

Cell imaging of apoptosis has been the subject of great interest in recent years as the activation or deregulation of programmed cell death plays a role not only during development but also in neoplasia and other medical disorders. Thus, the discovery of novel compounds that modulate apoptosis pathways could lead to the development of new therapeutic agents. This high content application can be a valuable tool in this context.

## References

Kerr JF et al, (1972): Apoptosis a basic biological phenomenon with wide ranging implications in tissue kinetics. *British Journal of Cancer*, 26, 239-257.

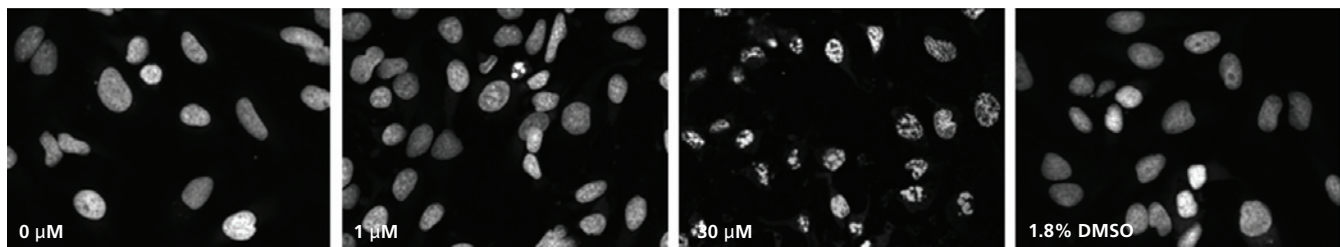
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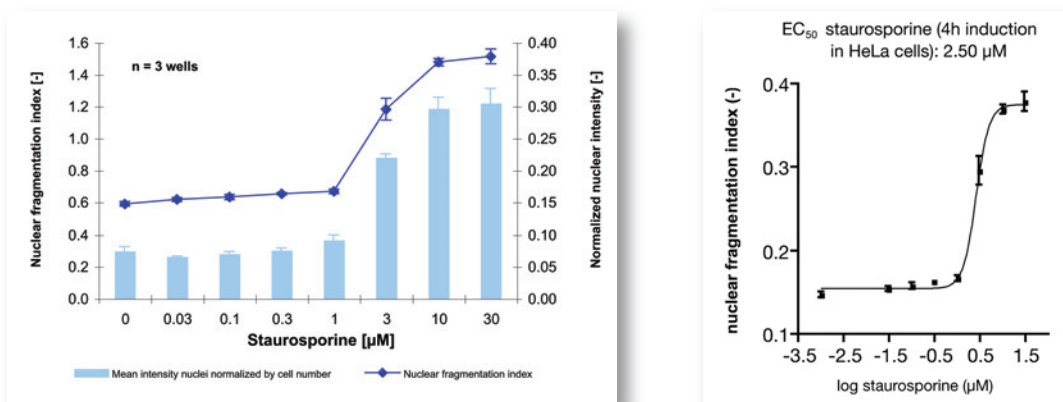


Figure 2: A. Confocal images of HeLa cells using the 40X water immersion objective. The nuclei display the nuclear phenotype associated with apoptosis: after shrinking, which condenses the nuclear stain onto a smaller area, the nuclei appear brighter; in a later stage of the apoptotic process the nuclei appear more fragmented (after treatment with 1, 3 and 30 μM staurosporine for 4 h). DMSO does not show any influence in the highest vehicle concentration used in this experiment.

B. Apoptosis manifests itself by an increasing level of nuclear fragmentation. In addition, observing the normalized nuclear intensity confirmed the course of the resulting dose-response curve. Fitting the nuclear fragmentation indices (with the Prism software) resulted in an EC<sub>50</sub> of 2.5 μM for staurosporine. Analysis was performed with images acquired with a 20X water immersion objective.

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