

Cellular Imaging and Analysis



Brightfield Image Acquisition

Digital Phase Image

Key Features

- Construction of digital phase images using the Opera system's brightfield option
- Single cell detection in digital phase images
- Cell detection without cytoplasmic marker

Digital Phase Imaging: Cell Detection and Analysis without Fluorescent Staining using the Opera High Content Screening System

Background

Image segmentation is a key aspect of high content screening (HCS) assays and involves separating objects of interest from background. This often requires using fluorescent stains to delineate the whole cell, allowing image segmentation into single cells. This technique, however, has a number of drawbacks such as the limited number of available fluorescent channels, overlapping excitation and emission spectra of dyes, and phototoxicity if the dyes are applied in live cell experiments [Selinummi *et al.*, 2009]. These difficulties can be overcome to some extent by using the brightfield option in HCS approaches.

By using a red light-emitting diode (LED), image acquisition with low phototoxicity can be achieved. The resulting brightfield images contain valuable information that can be used to distinguish cell regions from background by applying texture based segmentation methods [Operetta Application Guide, Ali *et al.*, 2010]. Such basic segmentation can be useful for applications such as proliferation or migration assays or for cell culture quality control by analyzing the confluency of cell monolayers. However, the segmentation of brightfield images is error prone, particularly with regard to detecting single cells. This is because the overall intensity of the background is approximately the same as the intensity of the cell.

Using the brightfield option of the Opera® High Content Screening System, a digital phase image can be constructed using two brightfield images acquired at different Z planes. The digital phase image generated has comparable properties to an image showing fluorescently labeled cells with high signal to noise ratios and allows image segmentation into single cells.

Application

To compare the image segmentation and analysis results of digital phase images with their corresponding fluorescence images, DRAQ5™-labeled HeLa cells were chosen as a model system. HeLa cells were seeded at 2000, 4000 and 8000 cells per well into a 384-well CellCarrier™ microtiter plate (PerkinElmer, 6007550) and cultured overnight. Cells were fixed with 3.7% formaldehyde and stained for 1 hr using 10 μM DRAQ5™. Brightfield and fluorescence images were acquired on the Opera system using the 20X water objective.

As a basis for the construction of a digital phase image, two brightfield Z planes of certain heights need to be acquired. The suitable Z heights depend on the specimen thickness as well as the objective magnification. As a first step, the appropriate Z planes need to be determined in one well by acquiring a stack and applying the “Z plane selection” script. For the Z plane selection in this example, a stack measurement of 55 planes with a distance of 0.9 μm was acquired. The “Z plane selection” script selected two planes, 18 μm and -15 μm , which were most appropriate for digital phase image generation, resulting in images with high signal to noise ratio (Figure 1). To achieve a good sampling, a plane distance of half the focal depth of the respective objective should be used. The appropriate focal depth values for all available objectives can be found in the Opera Application Guide. As a second step, the calculated Z planes can then be used for the whole plate measurement.

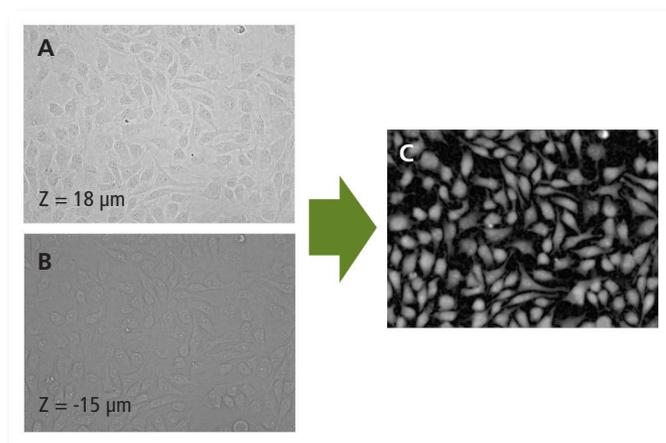


Figure 1. Construction of a digital phase image. Left: A suitable pair of brightfield images (HeLa cells) acquired using the 20X water objective at two different Z heights (panel A, 18 μm and panel B, -15 μm). Right (C): Corresponding digital phase image generated by the Acapella script “Z plane selection”.

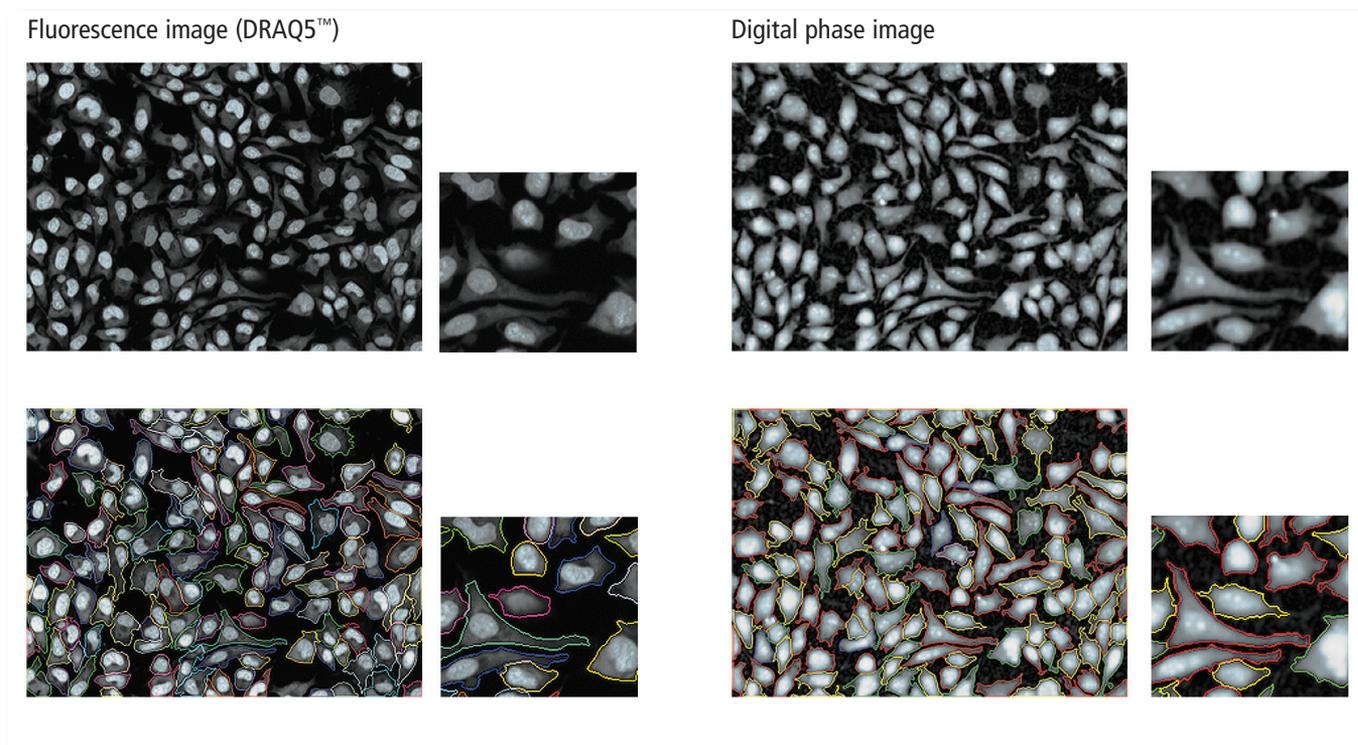


Figure 2. Comparison of image segmentation using either a fluorescence image (left 4 panels) or a digital phase image (right 4 panels). Non-segmented images (upper panels) and respective segmented images (lower panels) are shown with corresponding enlarged image sections. The sample image shows DRAQ5™-labeled HeLa cells with a seeding density of 8000 cells per well.

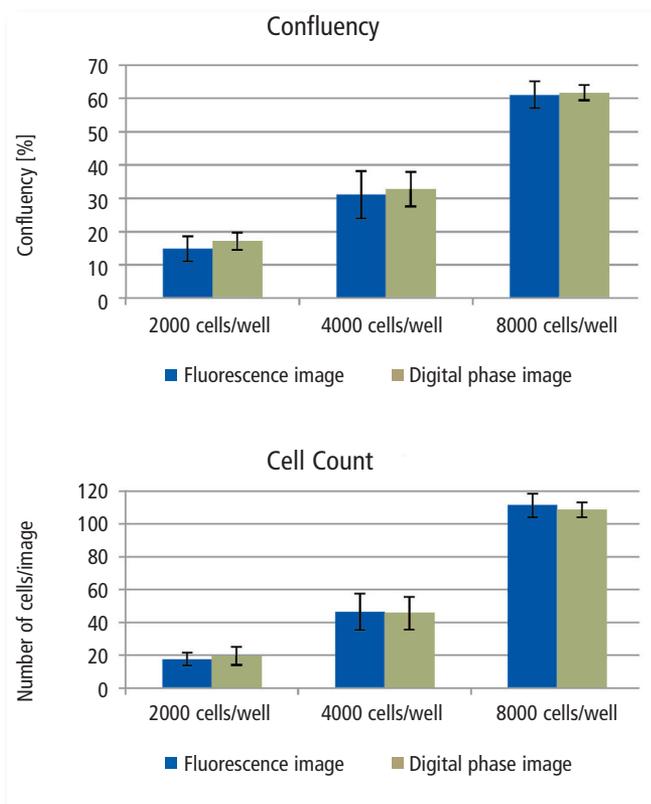


Figure 3. The segmentation of either fluorescence images or generated digital phase images leads to comparable cell counts and confluency levels at three different cell densities. (n=8 wells).

The digital phase image construction takes a computational approach for the generation of phase images based on brightfield images. The algorithm essentially calculates the rate of change in light intensity distribution between both brightfield images, which is introduced by changes in the refraction index of the specimen.

Cells in the digital phase image have high resemblance to fluorescently labeled cells (Figure 2) and image segmentation using the Acapella script “detect unstained cells” results in comparable readouts for Confluency and Cell Count (Figure 3). The script enables tuning of background correction as well as setting thresholds for cell detection and cell splitting, ensuring reliable image segmentation even at high cell densities.

Conclusions

In this study, we have introduced the construction of digital phase images using the brightfield option of the Opera system. The brightfield option uses a red LED and therefore image acquisition, especially for live cells, can be achieved with low phototoxicity. We showed that the single cell detection on a digital phase image is reliable and displays similar results when compared with segmentation based on a fluorescence image. Even cells at higher cell densities can be separated. Additional cytoplasmic staining is redundant and the free fluorescence channel can be used for other markers of interest. As a result, digital phase imaging reduces both the experimental complexity and costs, and can be considered a substantial advancement in the field of High Content Analysis.

References

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