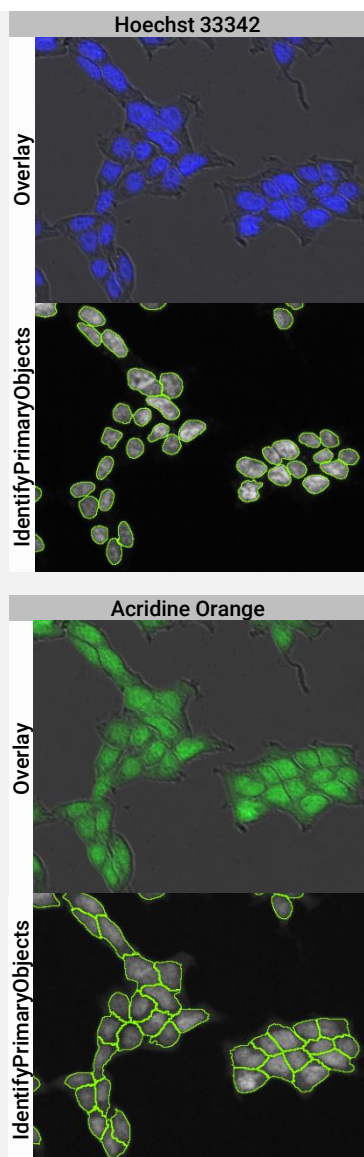


# Counting adherent cells in multi-well plates with the CELENA® X



**Figure 1.** Identification of adherent cells for high content cell counting. Example images of HEK293 cells with nuclei labeled with Hoechst 33342 and acridine orange and then segmented with Cell Analyzer software using the *IdentifyPrimaryObjects* module.

## INTRODUCTION

Cell-based assays are foundational to drug discovery and development studies. Serving as a way to normalize experimental conditions and data as well as monitor cell health, cell counting is an important quality control parameter for such assays.

The CELENA® X High Content Imaging System is convenient for capturing and analyzing images of adherent cells directly from well-plates to determine cell counts in a high content capacity. The CELENA® X can be set up to image multi-well plates in up to four fluorescence channels and is integrated with CELENA® X Cell Analyzer software that can assess captured images for multiple parameters and cellular phenotypes. In this study, we investigated cell counting capabilities of the CELENA® X by double staining HEK293 cells with acridine orange and Hoechst 33342.

## APPLICATION

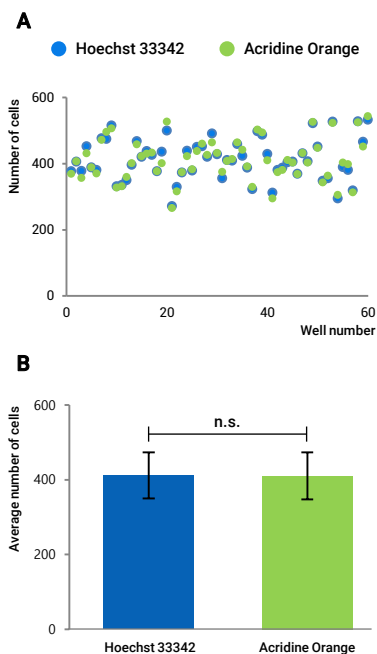
### Cell preparation

HEK293 cells were counted with the LUNA-II™ Automated Fluorescence Cell Counter, seeded at a density of  $1 \times 10^4$  cells/well into a 96-well plate, and cultured overnight. Cells were washed with PBS, fixed in 100  $\mu$ L 4% PFA for 15 minutes at room temperature, and then washed twice with PBS. For cell staining, 100  $\mu$ L acridine orange (Logos Biosystems, F23002) was added to each well and incubated for 15 minutes at room temperature. After washing each well twice with PBS, 100  $\mu$ L of 4  $\mu$ g/mL Hoechst 33342 (Life Technologies, H3570) was added to each well and incubated for 15 minutes at room temperature. Cells were washed twice in PBS to remove excess stain before imaging.

### Imaging and analysis

Brightfield, GFP, and DAPI images were acquired from 60 wells of the 96-well plate with the CELENA® X High Content Imaging System using a 10X LWD high NA objective and image-based autofocus.

Cell counting with CELENA® X Cell Analyzer software is based on identifying nuclei or cells with the *IdentifyPrimaryObjects* module from a designated channel. Nuclei are easily identifiable with this module as they are uniform in shape, have a high contrast relative to its background once stained, and are well-spaced apart from adjacent nuclei. Although both



**Figure 2.** Cell numbers produced by the IdentifyPrimaryObjects module. (A) Hoechst 33342 and acridine orange staining resulted in the same or a similar number of cells being counted in each well. (B) When the number of cells in each well were averaged, there was no significant difference seen for the two staining methods (n.s., not significant).

acridine orange (GFP) and Hoechst (DAPI) are fluorescent dyes that stain nuclei, Hoechst distinctly stained nuclei whereas acridine orange was also seen diffusely throughout the cytoplasm. To identify the cells stained with each dye, two *IdentifyPrimaryObjects* modules were used in sequence. Table 1 shows the recommended adjustments to make to the *IdentifyPrimaryObjects* module for each dye.

**Table 1.** Description of the IdentifyPrimaryObjects advanced settings used to identify cells and determine cell number.

	Hoechst 33342	Acridine Orange
Channel	DAPI	GFP
Threshold strategy	Adaptive	Adaptive
Threshold method	MCT	Otsu
Size of smoothing filter	15	14
Suppress local maxima	5	10

CONCLUSION

In this study, we demonstrated how simple it is to count adherent cells with the CELENA® X High Content Imaging System. Using the CELENA® X for fluorescence cell imaging paired with automated object identification and quantification gives researchers a simple and flexible way to monitor diverse cell-based assays. The same analysis pipeline can be reused to verify or compare results, making the CELENA® X a powerful tool for cell-based assays.

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