APPLICATION NOTE

# Count cells with or without fluorescent labels using automated imaging

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## Introduction

The ability to accurately quantitate cell number in multi-well microplates enables a multitude of biological applications that study cell health or proliferation. These applications may make use of endpoint assays for imaging fluorescently stained nuclei or may demand robust transmitted light imaging of unstained live or fixed cells. In both cases, the enumeration of the cells through software segmentation should be fast and reliable. The ImageXpress® Pico Automated Cell Imaging System with CellReporterXpress™ Image Acquisition and Analysis Software is ideal for quantitating cells whether label-free or fluorescently stained. In this application note, we demonstrate how the user's choice of transmitted light segmentation (analysis) algorithms increase the accuracy of counting diverse cell types and compare the results to those found when using a nuclear stain.

## Methods

In the following experiments, several cell types were plated into 96 well plates in 1:2 serial dilutions and grown overnight before being labeled with 5 µM Hoechst or DRAQ5 nuclear stain for 30-60 minutes inside an incubator at 37°C, 5% $CO_2$. Plates were read on the ImageXpress Pico system with a 4x or 10x Plan Fluor objective, one field of view/well. Fluorescent and transmitted light images were acquired consecutively (transmitted light first) and cells were counted using on-the-fly analysis. On-the-fly analysis allows for simultaneous analysis of an image during acquisition.

## Benefits

- **Use fluorescently stained or unlabeled cells**
- **Count multiple cell types and sizes**
- **Identify pipetting artifacts or non-uniform cell growth prior to running assay**

### Cell counting using ImageXpress Pico system

Add dye to the samples (this step may be omitted if doing label-free counting)

Place the plate into the ImageXpress Pico system and set up an acquisition using a preconfigured protocol

Review results determined automatically by the system

## Select the best transmitted light module for your cells

Three modules are included in CellReporterXpress software for counting cells in transmitted light. “Transmitted Light Cell Count, General” works well for most monolayer cell culture (CHO, Hela, PC-12) that is neither too confluent nor too sparse. In the experiments reported in Figure 1, however, cells were plated into 96 well plates from fully confluent to only a few cells per well. The corresponding TL modules yielded cell counts that most closely agreed with the quantitation achieved using a nuclear stain over the widest range of cell densities. For cells like HepG2 which grow in clumps, accurate segmentation can be challenging at high densities, so it may be advisable to use the measurement “Area covered” instead of “Cell count” to get more accurate results.

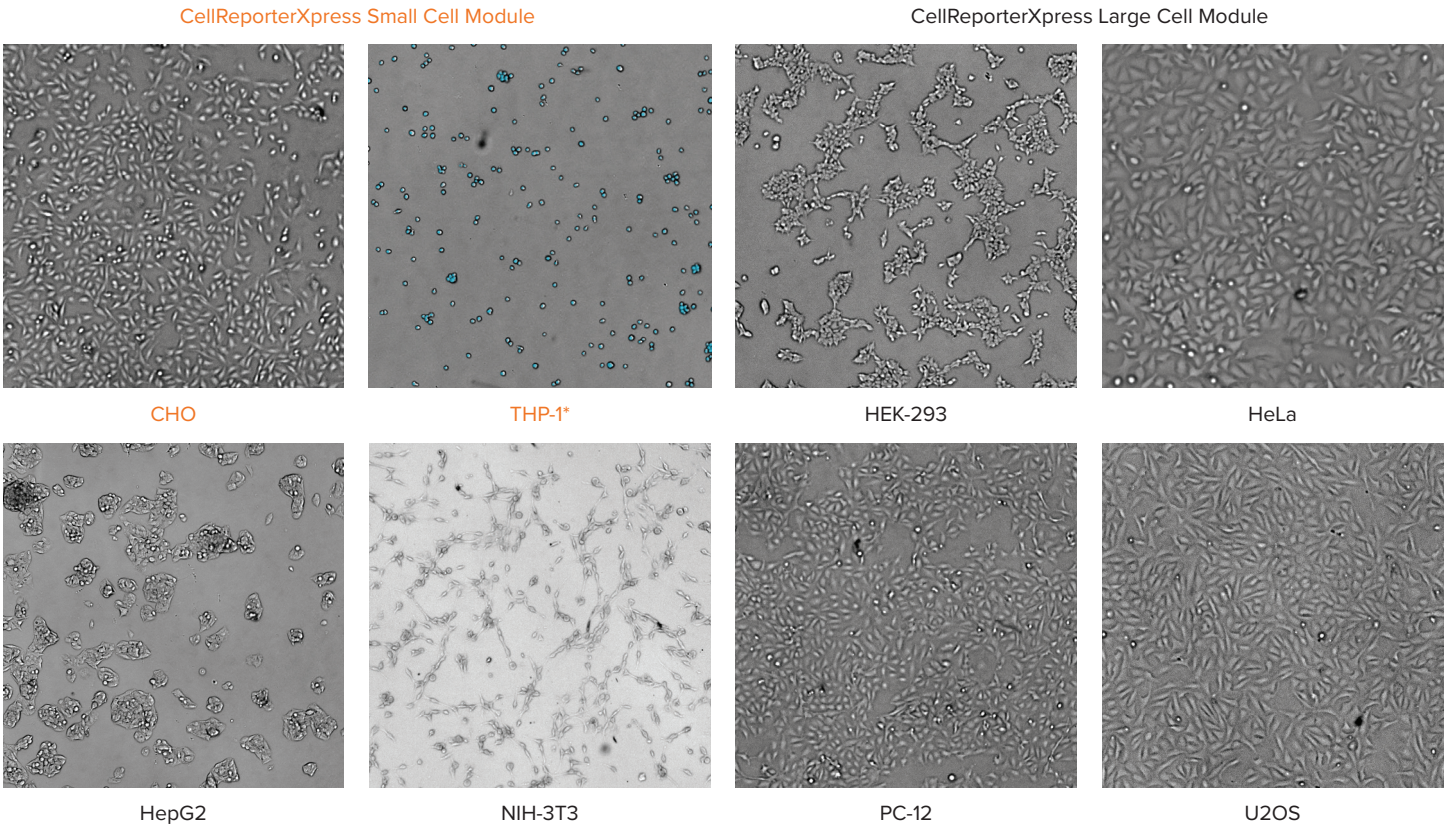



**Figure 1. Comparison of different cell types from 156-20,000 cells/well shows CellReporterXpress software modules that segmented the transmitted light images most accurately.** The transmitted light images were acquired at a z offset of -40 µm using a 10x objective. * THP-1 are suspension cells.

## Count adherent or suspension cells

Adherent monolayers or suspended cells could be quantitated using one of the three transmitted light (TL) cell counting analyses protocols based on cell size or the Cell Count module for detecting fluorescently stained nuclei (Figures 2 and 3). A comparison of cells detected using stained nuclei vs. unlabeled cells was consistent across wells that were not overly-confluent (Figure 4).

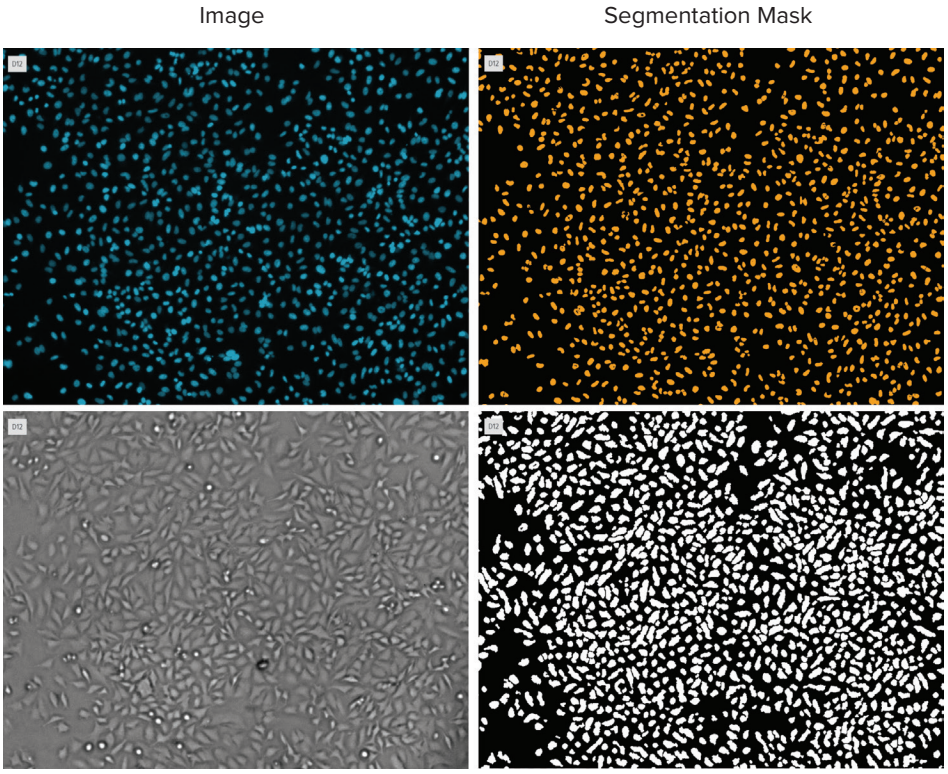



**Figure 2. Comparison of cell count on HeLa cells using fluorescence and transmitted light.** Top images show adherent HeLa cells acquired with a 10X objective on the left (blue nuclear stain) and the resulting analysis mask (orange) on the right. The lower panel shows the same region imaged with transmitted light **(left)** and the resulting analysis segmentation (white) on the right. Cell total was 1710 using nuclear count vs. 1790 using the transmitted light image.

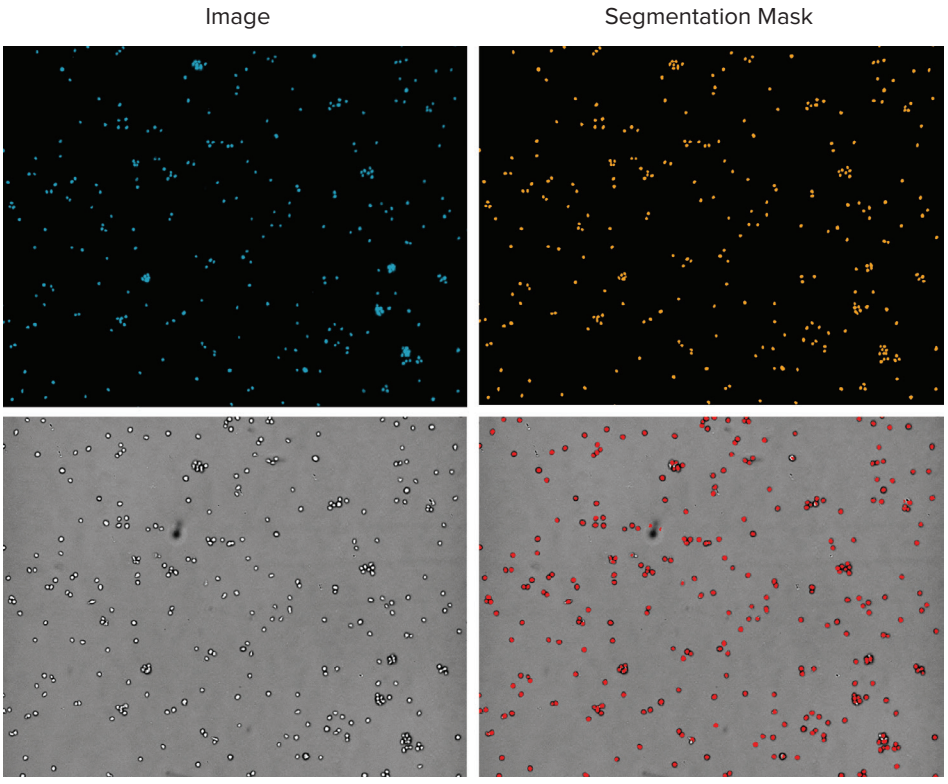



**Figure 3. Comparison of suspension cells using fluorescence and transmitted light.** Top images show THP-1 leukocytes acquired with a 10X objective on the left (blue nuclear stain) and the resulting analysis mask (orange) on the right. The lower panel shows the same region imaged with transmitted light **(left)** and the resulting analysis segmentation (red) on the right. Cell total was 325 using nuclear count vs. 320 using the TL image.

### Detect pipetting artifacts using whole plate thumbnail images

The entire plate can be scanned at low magnification in transmitted light (or fluorescence, if stained). Wells that demonstrate non-uniformity in the cell layer can be visually identified or detected on-the-fly using CellReporterXpress software (Figure 5).

## Conclusion

The ImageXpress Pico system allows researchers to quantitate a wide variety of live or fixed cells in a logical workflow that supports on-the-fly analysis with the accompanying CellReporterXpress software. Using the low magnification scan method enables an instant quality check of plating uniformity. In addition, observing effects on cell density due to compound treatment or reagent addition add a higher level of validation to experimental results.

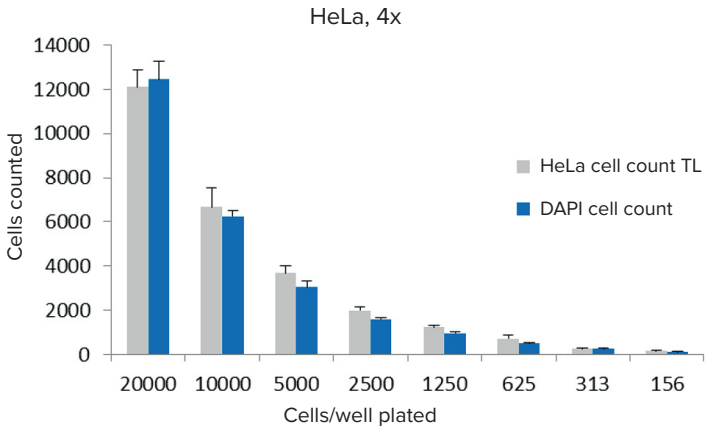
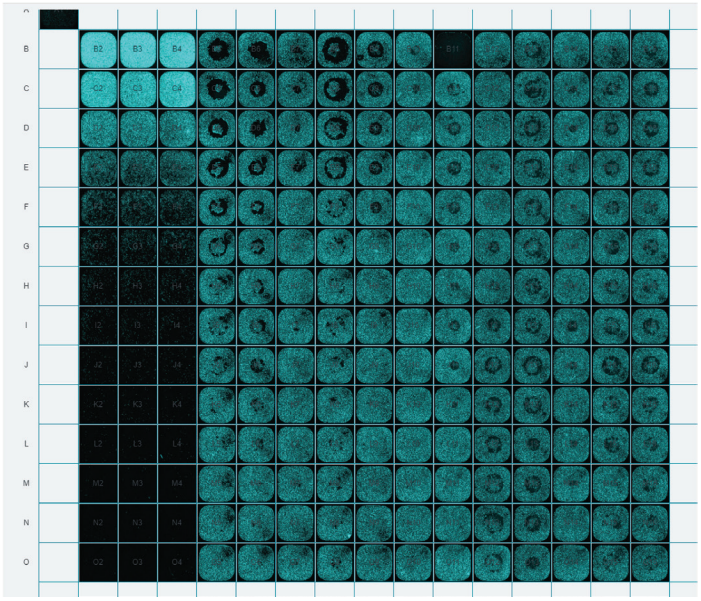



**Figure 4. A comparison of cell quantitation using transmitted light segmentation vs. Hoechst stained nuclei.** A single image/well was acquired (n=6 wells) using a 4x objective and analysis was performed using CellReporterXpress software preconfigured modules.



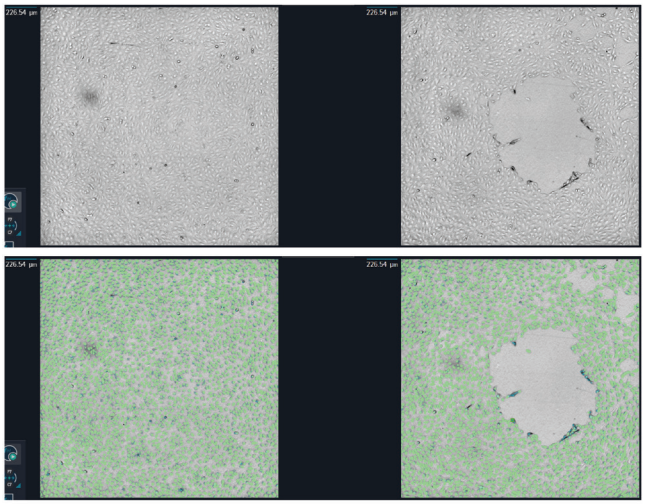


**Figure 5. Identifying pipetting artifacts using low magnification scan of an entire plate. (Left)** Plate montage view of Hoechst stained nuclei. Pipetting artifacts are readily apparent in the montage of this 384 well plate imaged at 4X. **(Right)** Transmitted light images (above) can be segmented and the analysis results (green mask overlay below) will highlight wells that do not contain a uniform number of cells.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