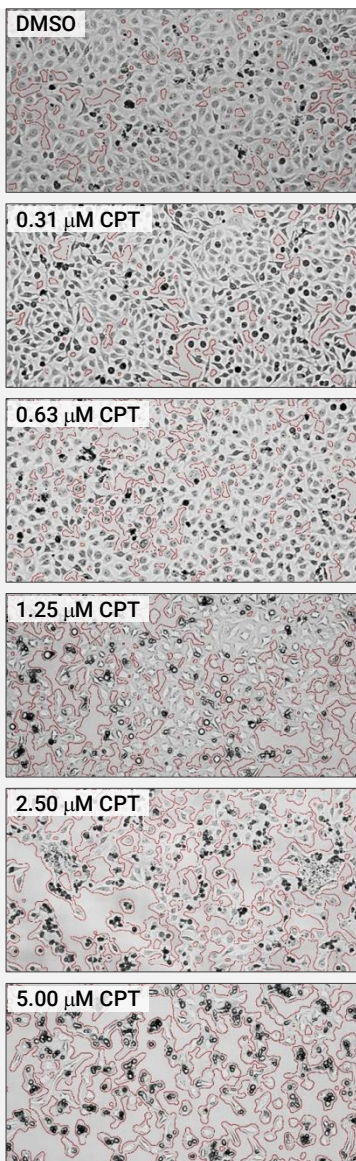


# Non-destructive quantification of cytotoxicity in live HeLa cells using the CELENA<sup>®</sup> X



**Figure 1.** Image analysis of HeLa cells after 16 hours of CPT treatment. Red borders illustrate the separation between background and the areas covered by intact cells. Image processing and object identification modules were applied to determine confluency.

## INTRODUCTION

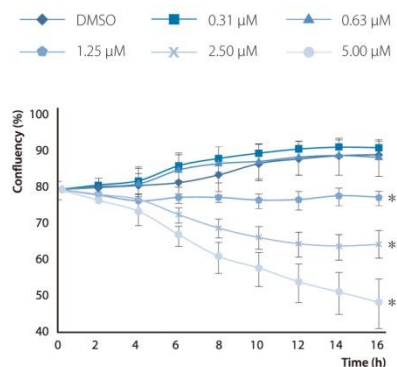
Cytotoxicity assays are a crucial step in screening for and developing therapeutic anti-cancer drugs. Most assays designed to measure cytotoxicity *in vitro* evaluate cell membrane integrity or metabolic activity after exposure, but are typically based on studying a single time point and require disturbing the growth of cells in culture.

Here, we demonstrate an automated, non-destructive method to monitor and quantify cytotoxicity based on its effects on confluency. This method uses brightfield imaging, which avoids the use of fluorescent stains that can have toxic effects in and of themselves over long incubation times. In this study, a combination of digital brightfield imaging with high content analysis using the CELENA<sup>®</sup> X High Content Imaging System provides quantitative readouts for assessing and comparing confluency changes over time.

## APPLICATION

To study cytotoxicity *in vitro*, we treated HeLa cells with different concentrations of Camptothecin (CPT; Abcam, ab120115). HeLa cells were counted with the LUNA-II™ Automated Cell Counter, seeded at a density of  $1 \times 10^4$  cells/well of the 96-well plate, and cultured overnight. Cells were then treated with serial dilutions of Camptothecin (DMSO, 0.3125  $\mu$ M, 0.625  $\mu$ M, 1.25  $\mu$ M, 2.5  $\mu$ M, and 5  $\mu$ M) at eight wells per condition and placed in the CELENA<sup>®</sup> X onstage incubator with 95% humidity and 5% CO<sub>2</sub> at 37°C. Cells were imaged automatically at two-hour intervals for 16 hours with the CELENA<sup>®</sup> X High Content Imaging System using a 4X LWD objective and image-based autofocus. Acquired images were analyzed using CELENA<sup>®</sup> X Cell Analyzer software.

The strategy for confluency analysis with the Cell Analyzer software is based on identifying cells and measuring the total area occupied by these objects. A pipeline was created in Cell Analyzer to automatically batch process and analyze images. To do this, the *EnhanceEdges* module was used to create binary images that distinguished the foreground (the cells) from the background. The *Smooth* module was used to reduce intensity irregularities, which homogenized the cells, smoothed edges, and removed debris from the background. The resulting segmented areas were identified as cells using the *IdentifyPrimaryObjects* module and



**Figure 2.** Dose-response curves deduced from confluency. A significant loss of cells was observed with increasing concentrations of CPT. N =8 wells. \* p : <0.05.

then measured using the *MeasureImageAreaOccupied* module to quantify the surface area occupied by the cells within the field. The *OverlayOutlines* module was used to overlay the original brightfield images with the outlines of the segmented areas to visually confirm segmentation precision.

Figure 1 shows the segmentation of brightfield HeLa cell images after 16 hours of CPT treatment. Image segmentation using the created Cell Analyzer pipeline showed that compared to the control group, there was a 12.8%, 27.1%, and 44.6% reduction in confluency after 16 hours of incubation with 1.25 µM, 2.5 µM, and 5.0 µM CPT, respectively, whereas incubation with 0.3125 µM and 0.625 µM had negligible results (Figure 2).

## CONCLUSION

The determination of cytotoxicity is essential to drug development. In this study, we analyzed the effects of Camptothecin on confluency using the CELENA® X High Content Imaging System. Live cell imaging combined with analysis of cell surface area occupying each well is a reproducible and quantifiable approach for measuring cytotoxicity. The easy-to-use, powerful building blocks of the Cell Analyzer software provides a simple way to quantify confluency objectively.

For the cytotoxicity study here, the measurement of confluency as an analysis tool for cytotoxicity was effective, simple, and most importantly, highly reproducible. Using the CELENA® X High Content Imaging System and CELENA® X Cell Analyzer software, the same experimental conditions as well as the identical analysis pipeline can be reused to verify results.

## REFERENCES

- Ding X, Matsuo K, Xu L, Yang J, Zheng L. 2015. Optimized combinations of bortezomib, camptothecin, and doxorubicin show increased efficacy and reduced toxicity in treating oral cancer. *Anticancer Drugs*. 26(5): 547-54.
- Giovanella BC, Stehlin JS, Hinz HR, Kozielski AJ, Harris NJ, Vardeman DM. 2002. Preclinical evaluation of the anticancer activity and toxicity of 9-nitro-20(S)-camptothecin (Rubitecan). *Int J Oncol*. 20: 81-8.
- Takasuna K, Kasai Y, Kitano Y, Mori K, Kobayashi R, Hagiwara T, Kakhata K, Hirohashi M, Nomura M, Nagai E, Kamataki T. 1995. Protective effects of kampo medicines and baicalin against intestinal toxicity of a new anticancer camptothecin derivative, irinotecan hydrochloride (CPT-11), in rats. *Jpn J Cancer Res*. 86: 978-84.
- Wang MJ, Liu YQ, Chang LC, Wang CY, Zhao YL, Zhao XB, Qian K, Nan X, Yang L, Yang XM, Hung HY, Yang JS, Kuo DH, Goto M, Morris-Natschke SL, Pan SL, Teng CM, Kuo SC, Wu TS, Wu YC, Lee KH. 2014. Design, Synthesis, Mechanisms of Action, and Toxicity of Novel 20(S)-Sulfonylamidine Derivatives of Camptothecin as Potent Antitumor Agents. *J Med Chem*. 57: 6008-18.
- Yount G, Soroceanu L, Wang HJ, Singer E, Yount R, Luu T, Yang LX. 2016. Selective Toxicity of a Highly Potent Camptothecin Analogue: A Pilot Study with Glioblastoma Multiforme Cells. *Anticancer Res*. 36(1): 5845-48.
- Zhang L, Ma D, Zhang Y, He W, Yang J, Li C, Jiang H. 2013. Characterization of DNA topoisomerase-1 in *Spodoptera exigua* for toxicity evaluation of camptothecin and hydroxy-camptothecin. *PLoS One*. 8(2): e56458.