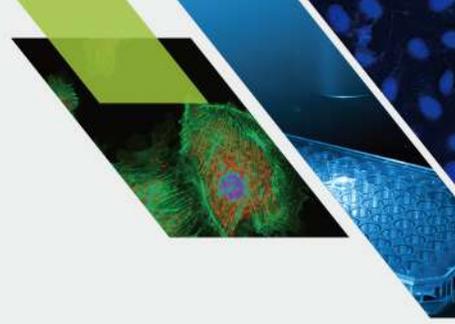
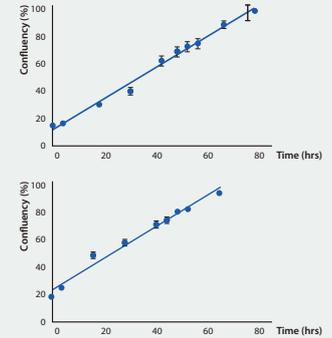
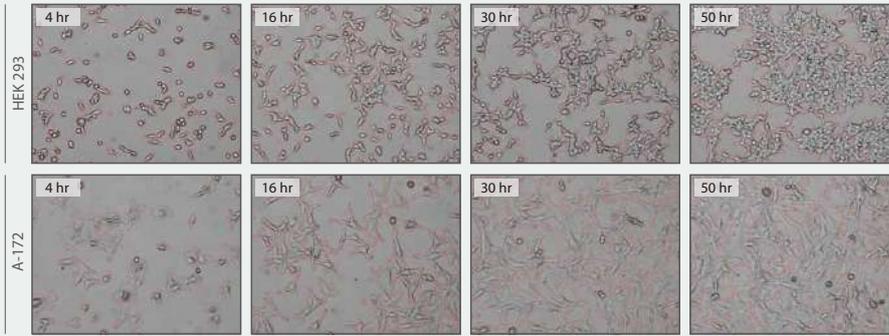


# Applications using the CELENA<sup>®</sup> X High Content Imaging System in cell biology



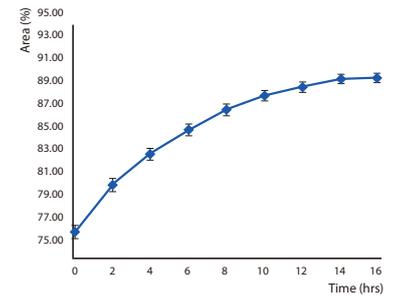
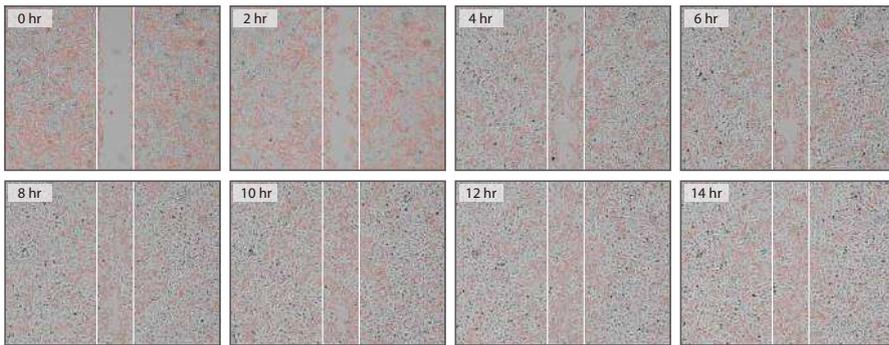
## CELL CONFLUENCY ASSAY



### Time-lapse measurement of cell confluency using CELENA<sup>®</sup> X

HEK293 and Glioblastoma A-172 cells were seeded in a multi-well plate, and the occupied cell area was monitored using CELENA<sup>®</sup> X Stage Top Incubation System. The cell images in the matching fields of the assigned wells were timely acquired in a non-invasive and label-free manner every four hours for 64 hours. The images were then processed to present cell boundaries with outlines and analyzed for cell growth rate. The selected cell images represent the edges of the cell area at the respective time points.

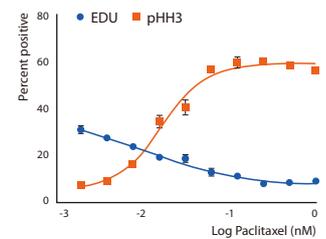
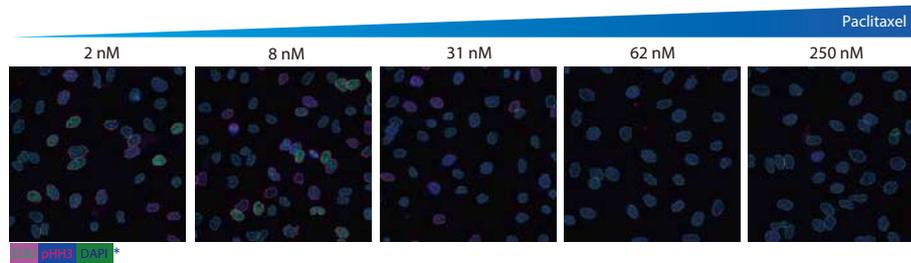
## WOUND HEALING ASSAY



### 2-D migration of wound healing assessment using CELENA<sup>®</sup> X

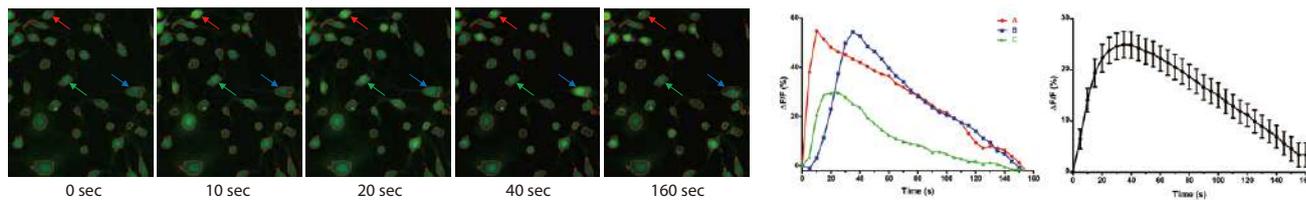
The bovine aortic endothelial cells (BAEC) were employed for the wound healing assessment with the CELENA<sup>®</sup> X Stage Top Incubation System. The linear wound area was created on the confluent six-well plate and screened at two hours intervals for 16 hours, and then the acquired images were batch processed to measure the wound closure area over time. The cell images were acquired the same fields of assigned wells over time by CELENA<sup>®</sup> X, and the time-lapse measurement of wound closure was accordingly analyzed and effortlessly processed as the cell migration assessment.

## CELL CYCLE ASSAY



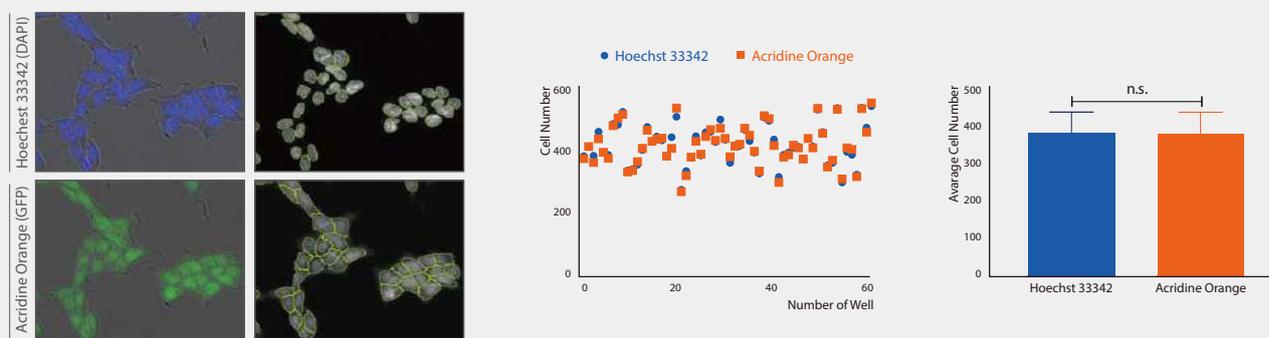
### Evaluations of cell cycle arrest by Paclitaxel with the CELENA<sup>®</sup> X

HeLa cells were treated with 5 doses of a mitotic inhibitor Paclitaxel for 16 hours. The cells were then double-labeled with EdU which is a mitotic marker for detecting newly synthesized DNA targeting on  $\alpha$ -phosphohistone H3 (pH3), and Hoechst33342 for DAPI signal detection in nuclei. The cell images were captured and batch processed with CELENA<sup>®</sup> X to quantify the integrated intensity of the multiplexed signals. The images represent the cells labeled in fluorescence with coordination of pseudo-colors and outlines for the respective dosage of Paclitaxel. The presented plot indicates the cell cycle arrest profiles in a dosage-dependent manner of Paclitaxel based on the combinations of the two signals.



**Fluorescence-based calcium mobilization assay with the CELENA® X**

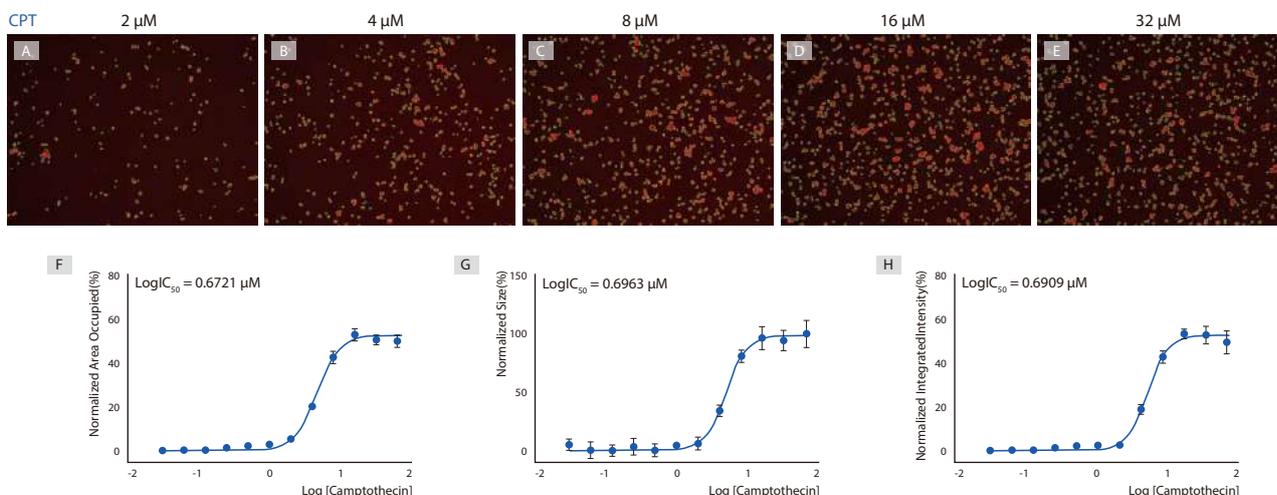
HeLa cells were applied for time-lapse measurement of ATP-dependent calcium efflux every 5 seconds for 160 seconds. The selected five frames of cell images exhibit the changes of Fluo-8 signal by calcium mobilization after treatment of 20 mM ATP reagent. The three arrows in the images were individually accessible for the changes of fluorescence signals over time and designated on the color-coordinated plot. The total changes of integrated intensity were plotted to demonstrate fluorescence-based calcium mobilization assay.



**Automated cell counting with multiplex fluorescent labeling using CELENA® X**

HEK293 cells in a 96-well plate were stained with Hoechst33342 for DAPI signals in nuclei and Acridine Orange for GFP signals in the cytoplasm. When the two fluorescent labeled cell images were automatically acquired, and the object identification and quantification were processed, the consistent number of cells were counted between the DAPI and the GFP images. The plots signify that the counting of nuclear and cytoplasmic markers has cross-referenced each other and the CELENA® X prodigiously accomplished the automated acquisition of cell images and batch processing for cell counting assays.

\* n.s., not significant ( $p < 0.01$ )



**High content cytotoxicity analysis of anticancer drug using CELENA® X**

HeLa cells were treated with 12 doses of Camptothecin (CPT) for 16 hours and subsequently stained with propidium iodide (PI) to investigate the CPT cytotoxicity in different drug concentrations. The red fluorescent (RFP) cell images were acquired by CELENA® X Explorer, and the CELENA® X Cell Analyzer was employed to batch process measurement of cell area (F), cell size (G) and integrated RFP intensity (H) to profile the CPT dependent cytotoxicity.