

Label-free cell imaging and analysis using transmitted light and deep learning

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Introduction

Label-free imaging offers significant advantages over traditional fluorescence-based methods, including reduced reagent costs, faster processing, and the ability to monitor live cells. Moreover, label-free detection offers advantages by enabling observation of biomolecular interactions without altering the sample, leading to more accurate data, deeper characterization, and real-time monitoring of kinetics. In this study, we used customizable AI models within IN Carta® Image Analysis Software to develop deep learning-based segmentation models for accurate cell and nuclear detection using transmitted light (TL) images alone. We began by establishing label-free analysis using the U2OS cell line. To develop a TL-based nuclear segmentation model, we first applied a pre-trained AI model to segment nuclei in Hoechst-stained images. The resulting segmentation masks, paired with corresponding TL images, were used to train a new model that segments nuclei using TL images only. This approach enabled label-free nuclear detection with over 97% accuracy, as validated against Hoechst-based nuclear counts. The model reliably quantified both live and damaged cell phenotypes. Additionally, we developed a whole-cell segmentation model trained on Calcein AM-stained images. This model also demonstrated high performance, achieving 97% accuracy compared to nuclear counts. We further trained the model using images from additional cell lines, including HeLa, HCT116, U2OS, MCF7, HEK, HepG2, MRC5, and DU145. The model achieved over 90% accuracy across most of these cell types. To evaluate its utility in compound screening, we treated these cell lines with 10 anti-cancer compounds using a 7-point dilution series. Cell counts obtained via TL-based nuclear segmentation were compared with those from traditional nuclear staining methods across treatments. The results showed strong correlation and comparable IC₅₀ values, confirming the accuracy and reliability of the label-free approach. ATP-based viability assays were performed in parallel, and a correlation was observed between ATP responses and label-free imaging-based phenotypic changes. Overall, this label-free approach offers significant advantages in processing speed and workflow simplicity, enabling real-time analysis of compound effects and making it a powerful tool for high-throughput and phenotypic screening applications.

Methods

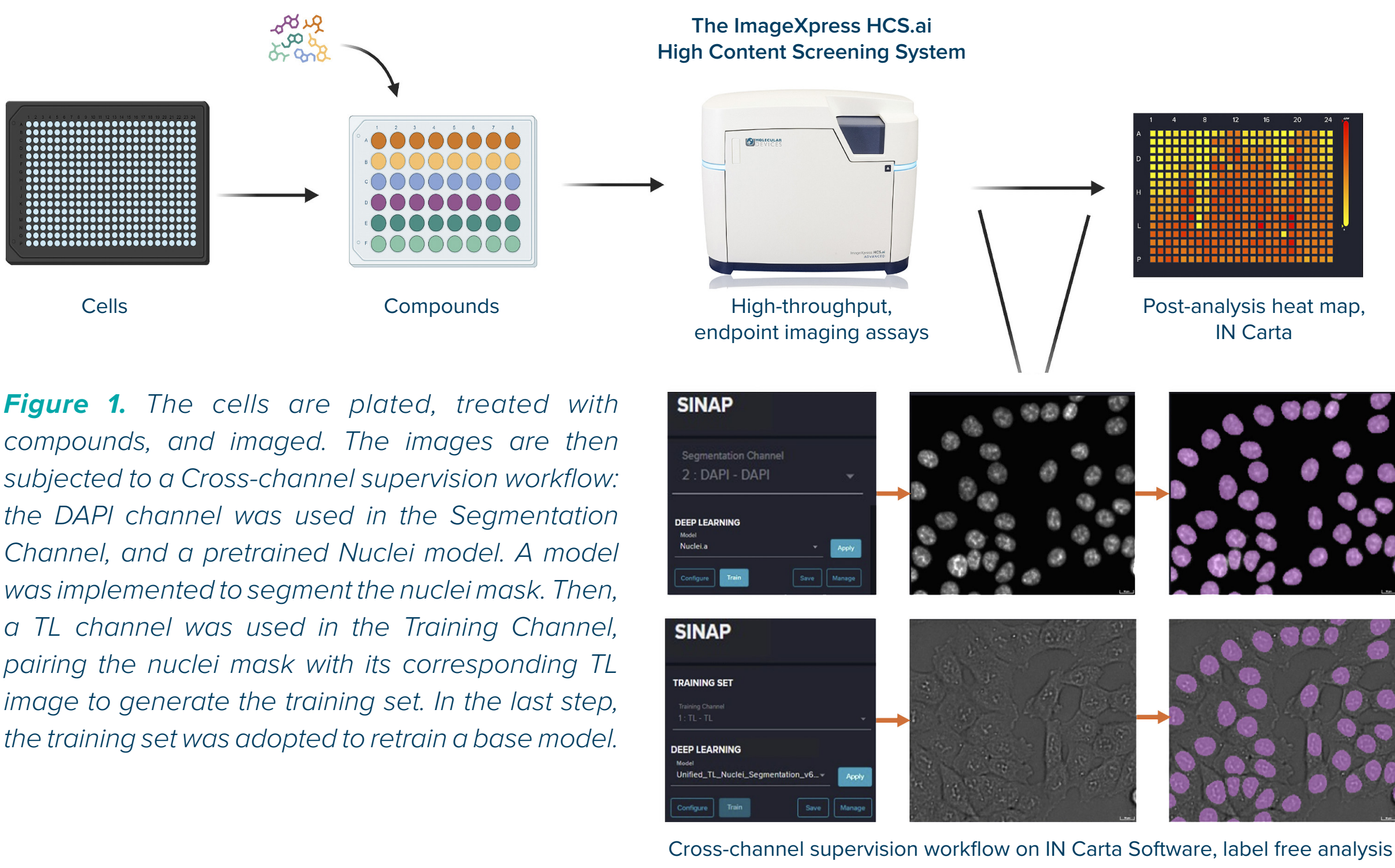
Cell seeding, treatment, and staining: For this experiment, U2OS, HCT-116, HeLa, HEK, and MCF-7 cells were seeded at 1,000 cells per well. After 24 hours, compounds (Staurosporine, Cytarabine, Etoposide, Doxorubicin, Rotenone, Carboplatin, Paclitaxel, SB202190, Rapamycin, and 5-Fluorouracil) were added at five-fold dilutions starting from 1 µM and treated for 72 hours. For cell staining, fluorescent dyes were added 20–30 minutes before imaging each plate. Final dye concentrations were approximately 0.75 µM Calcein AM, 1.5 µM EthD-1, and 7 µM DAPI for plates 2 and 3 (Figure 1).

Image acquisition and analysis: All plates were acquired using the ImageXpress® HCS.ai High-Content Screening System with a 10X objective. Images included four channels: Transmitted Light (TL), DAPI, FITC, and TX Red. Z-stacks spanning 50 µm at 5 µm intervals were collected, and 2D Best Focus projections were saved.

To develop a TL-based segmentation model for nuclei in IN Carta Software, a pre-trained artificial intelligence (AI) model segmented nuclei in Hoechst-stained images via the software's SINAP module. These masks, paired with TL images, trained the TL nuclei model (Figure 1). Similarly, for cell segmentation, a pre-trained model segmented cells in Calcein AM-stained images; masks and TL images were used to train the TL cell model.

After training, the TL nuclei model segmented nuclei (Nuclei), and the TL cell model segmented cells (Cells). A DAPI-based model provided ground truth (Nuclei_GT). Using a user-defined target, overlapping regions between Nuclei and Nuclei_GT were labeled Overlapping. These labels enabled performance metrics: Nuclei vs. Nuclei_GT for detection rate, and Nuclei vs. Overlapping for false positive rate. Finally, Nuclei counts were used to generate dose-response curves.

AI models were trained using fluorescence masks paired with TL images to segment nuclei and cells accurately. Integrated datasets from multiple cell lines improved model generalization. Custom targets enabled calculation of detection and false positive rates, achieving >90% detection and <2% false positives across most cell lines. Dose-response curves from TL-based counts strongly correlated with fluorescence-based counts, confirming reliability.



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Results

SINAP allows cross-channel supervision and a customizable AI model

Transmitted light (TL) microscopy images pose significant segmentation challenges due to low contrast and complex features, as they capture only light passing through the sample. Deep learning (DL) methods, particularly Convolutional Neural Networks (CNNs), are widely used but require careful optimization and curated training data. Manual annotation of TL segmentation is time-consuming and often inconsistent. To address this, we used the SINAP module in IN Carta Image Analysis software to customize AI models via cross-channel supervision-training masks from one channel while segmenting another (Figure 1). Using U2OS cells, we trained a TL nuclei segmentation model by generating DAPI-based masks with a pre-trained model and pairing them with TL images (40 mask-image sets). Similarly, Calcein AM masks were used to train a TL cell segmentation model.

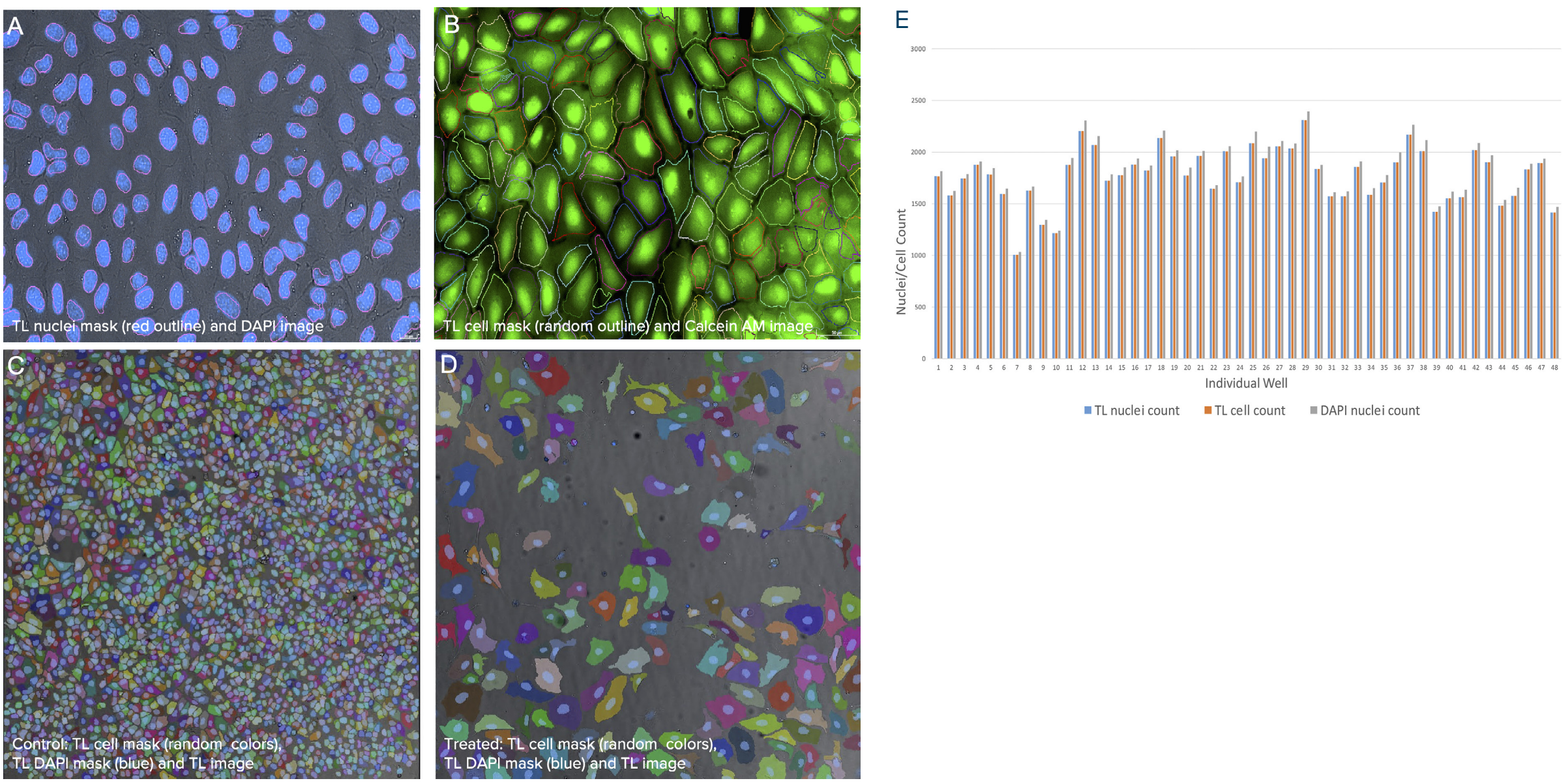


Figure 2. Illustrates the segmentation results from post-trained TL nuclei and TL cell models, along with nuclei and cell count comparisons using U2OS cells. Panel 2A shows TL nuclei segmentation masks (pink outlines) overlaid on DAPI-stained images (blue), confirming spatial alignment. Panel 2B displays TL cell segmentation masks (randomly colored outlines) overlaid on Calcein AM-stained images, validating the accuracy of cell segmentation. Panels 2C and 2D present overlays of TL cell masks (random colors) and TL nuclei masks (blue) on TL images from untreated and compound-treated wells, respectively. Panel 2E compares nuclei and cell counts across individual wells, demonstrating that TL nuclei counts achieved 97% accuracy with a standard deviation of 0.9% when compared to DAPI ground truth.

The Export/Import feature in SINAP enables multi-cell line model training

Dataset Integration for Model Generalization

Goal: Improve model performance across multiple cell lines by integrating diverse training datasets.

Tool: SINAP's EXPORT and IMPORT features were used to efficiently assemble data from five cell lines.

Two Approaches:

- Parallel** (Figure 3A): Export datasets from each cell line individually → Import collectively to create one combined dataset. Advantage: Reduces storage needs for large datasets.
- Sequential** (Figure 3B): Export all current datasets → Import into the following cell line → Build progressively. Advantage: Minimizes overhead when handling many cell lines.

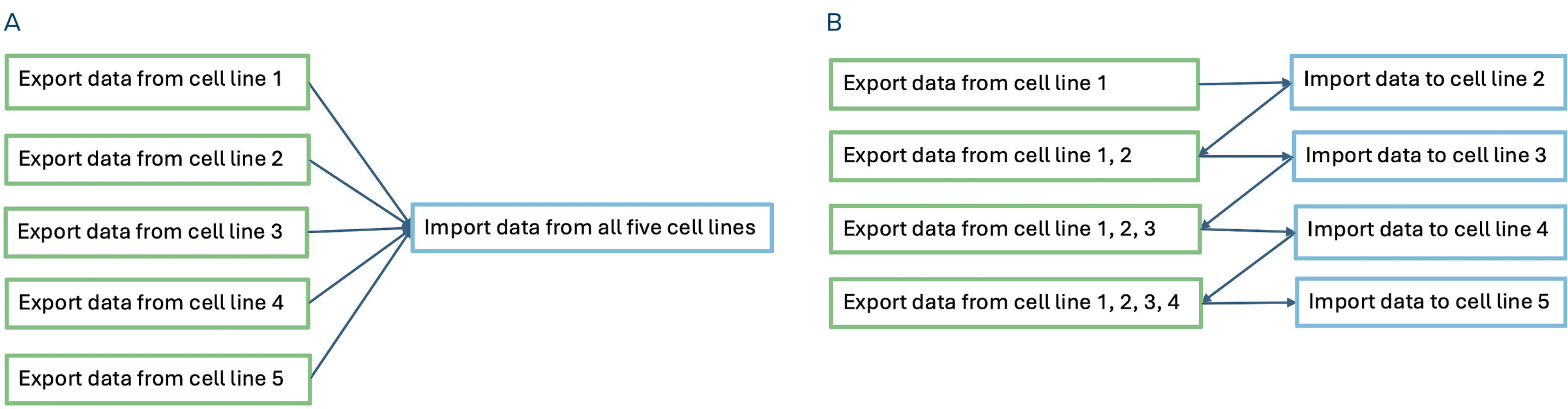


Figure 3. A. Workflow of exporting datasets in parallel. B. Workflow of export datasets sequentially.

SINAP's user-defined target feature enables measurement of the false positive rate

False Positive Rate Calculation

Metric: Proportion of actual negatives incorrectly classified as positives.

Steps:

- Segment nuclei from TL channel → label as Nuclei.
- Generate ground truth mask from DAPI channel → label as Nuclei_GT (Figure 4A).
- Use SINAP's User-Defined Target feature (supports AND, OR, NOT, XOR) → apply AND between Nuclei and Nuclei_GT → create Overlapping mask (Figure 4B).
- Link targets: Nuclei = Parent, Overlapping = Child → nuclei with zero overlapping child count = false positives.

Example: Overlapping mask generation shown in Figures 4C–4F.

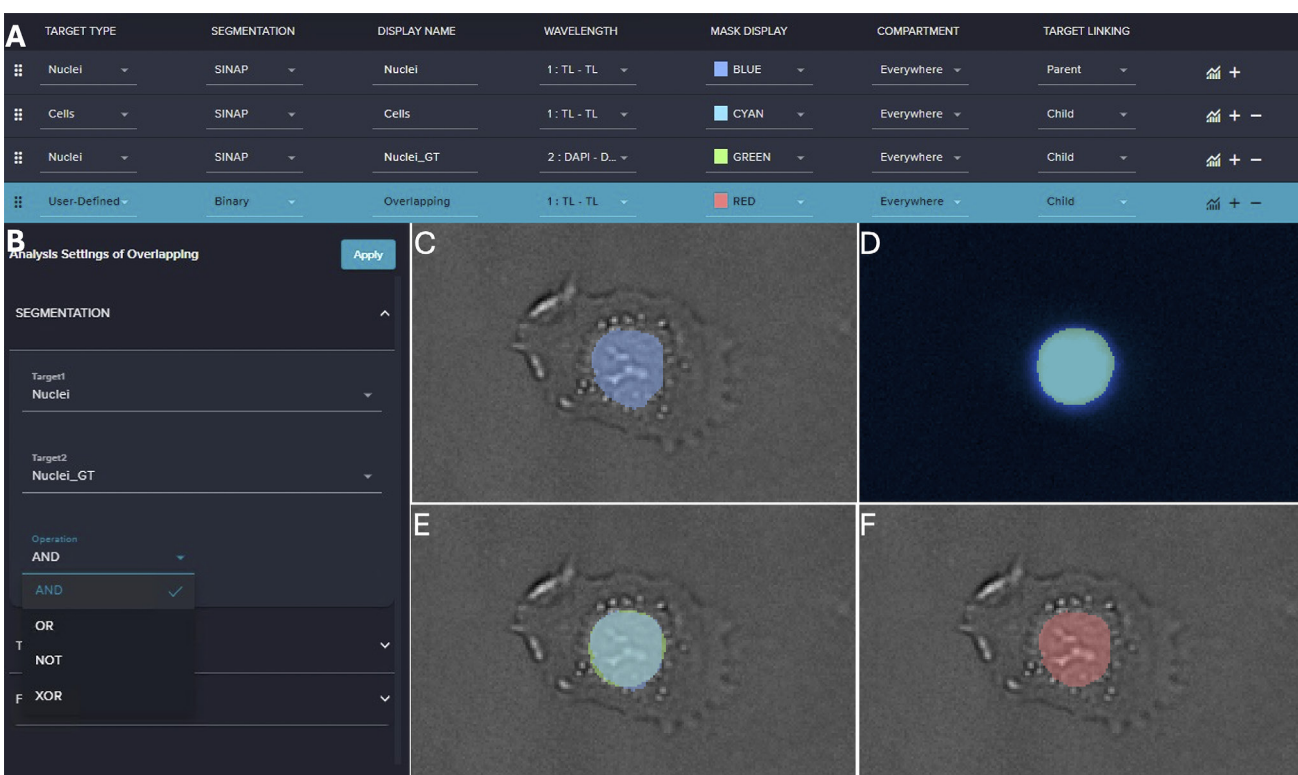


Figure 4. A. Screenshot of the IN Carta Software analysis workflow. B. Screenshot of setting User-Defined target. C. Overlapping of Nuclei (blue) mask and TL image. D. Overlapping of Nuclei (blue) and DAPI image. E. Overlapping of Nuclei (blue), Nuclei_GT (Green) and TL image. F. Overlapping of Overlapping (Red) and TL image.

The unified TL model detects nuclei in sync with DAPI signals

Unified TL Model Performance. The unified TL model detects nuclei in sync with DAPI signals. Detection and false positive rates were computed across cell lines. Most cell lines showed >90% detection, except HEK (~86%). For U2OS, accuracy was ~96%, slightly below the U2OS-specific model (97%) due to the influence from other cell lines (Figure 5A). False-positive rates were <2% in most cell lines, except for MCF-7 (~7%). Investigation revealed that MCF-7 clustering led to poor DAPI staining at cluster centers, impairing nuclear detection and increasing false positives. Dose-response curves from TL- and DAPI-derived counts showed strong correlation and comparable IC₅₀ values, confirming the accuracy and reliability of this label-free approach (Figures 6A–C).

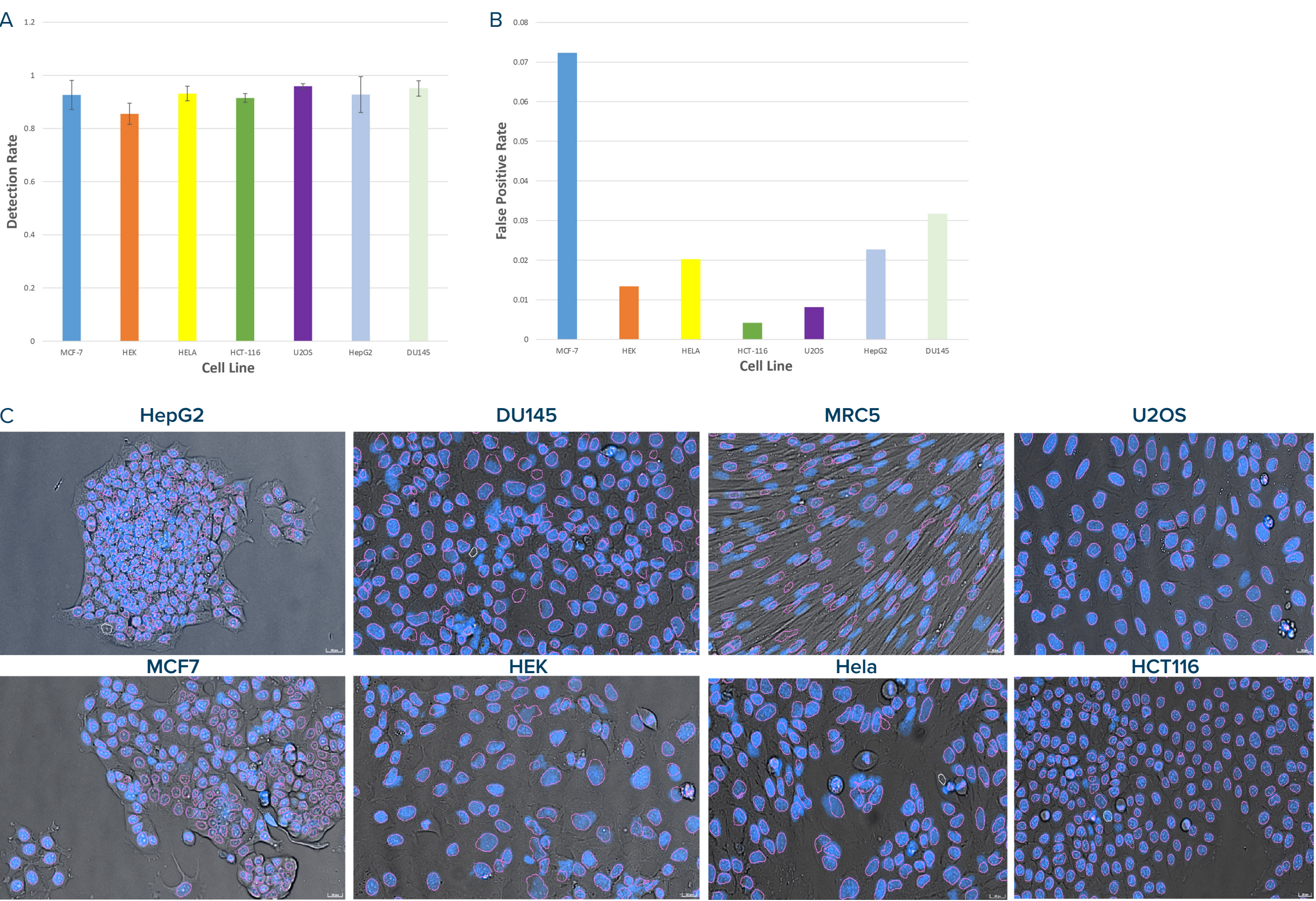


Figure 5. A. Detection rate of all cell lines. B. The false positive rate of all cell lines. C. Overlapping of DAPI nuclei mask (pink outline), DAPI image and TL image of all cell lines.

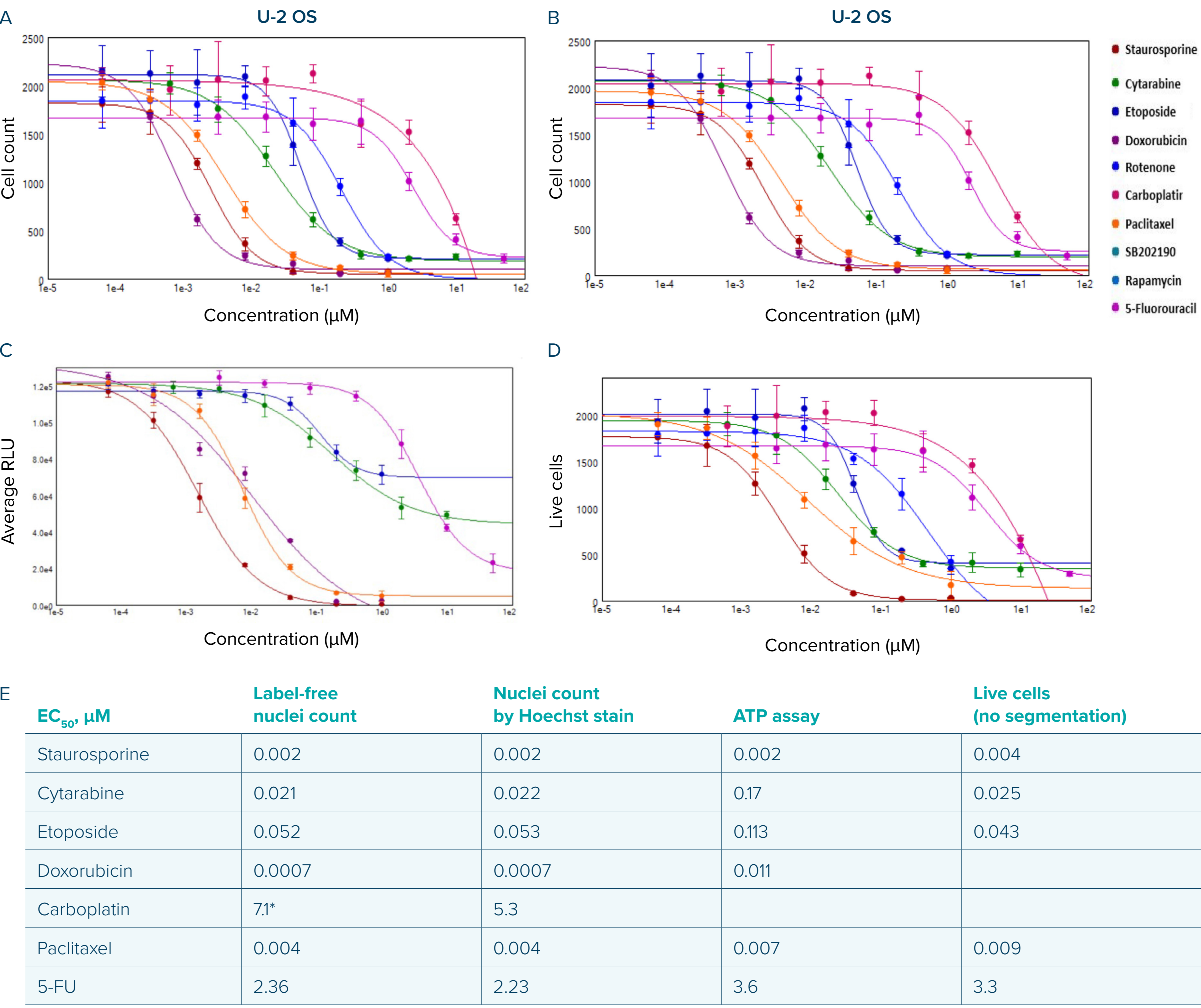


Figure 6. A. Dose-response curve of U2OS cells based on TL-derived nuclei counts. B. Dose-response curve of U2OS cells based on DAPI-derived nuclei counts (other cell lines exhibiting similar behaviors are not shown). C. Dose-response curve of U2OS cells based on ATP assay. D. Dose-response curve of U2OS cells based on Live cell count from simple threshold analysis. E. The EC₅₀ value comparison between TL-based nuclei counts, DAPI-based nuclei counts, ATP assay, and Live cell counts from simple threshold analysis. Cell count-based EC₅₀ values showed strong agreement across TL-derived and DAPI-based nuclei counts. ATP and Live Cell assays produced slightly shifted EC₅₀ values, reflecting the different nature of their readouts.

Summary

- Label-free imaging preserves cell viability and enables real-time, non-invasive monitoring of cellular dynamics.
- Paired with an AI model trained across diverse cell lines in IN Carta Software, this approach achieves high accuracy in detecting nuclei numbers.
- The AI model's customizable nature ensures scalability and relevance, and a user-defined target enables the integration of diverse measurement modalities.
- Overall, this platform paves the way for more efficient and insightful cell-based research.

