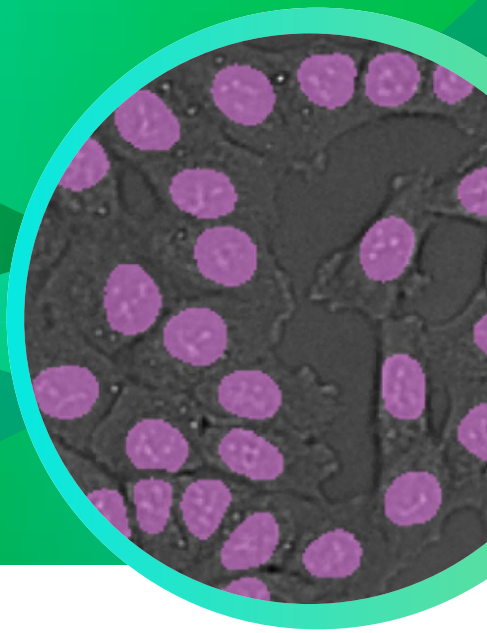
APPLICATION NOTE

AI-enabled label free detection with IN Carta Image Analysis Software

Zhisong Tong, PhD, Prathyushakrishna Macha, PhD, Oksana Sirenko, PhD | Molecular Devices, LLC



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 artificial intelligence (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 capable of segmenting 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.

Benefits

- Preserve cell viability and enables real-time, non-invasive monitoring of cellular dynamics by eliminating the need for fluorescent or chemical labels.
- Achieves high accuracy in nuclei number detection using AI models trained in IN Carta Image Analysis Software.
- Ensures scalability and relevance of AI model through customization and diverse measurement modalities through user-defined target.

We further trained the model using images from additional cell lines, including HeLa, HCT116, U2OS, MCF7, and HEK. The model achieved over 90% accuracy across most of these cell types. To evaluate its utility in compound screening, we treated the five cell lines with 10 anti-cancer compounds using a 7-point dilution series. Cell counts obtained via TL-based nuclear segmentation were compared to traditional nuclear staining methods across treatments. The results showed strong correlation and comparable IC_{50} values, confirming the accuracy and reliability of the label-free approach.

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 culture and treatment

For this experiment, U2OS, HCT-116, HeLa, HEK and MCF-7 cells were seeded at 1,000 cells per well. Compounds(Staurosporine, Cytarabine, Etoposide, Doxorubicin, Rotenone, Carboplatir, Paclitaxel, SB202190, Rapamycin and 5-Fluorouracil) were added 24 hours later. After 72 hours of treatment, the drugs were added in quadruplicates, at five-fold dilutions with 1µM starting concentration.

For staining of cells, 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 (Figure 1).

Image acquisition and analysis

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

To develop a transmitted light-based segmentation model of nuclei in IN Carta Image Analysis Software, we initially employed a pre-trained AI model to segment nuclei in Hoechst-stained images. The resulting segmentation masks, paired with their corresponding TL images, were then used to train a new model capable of segmenting nuclei directly from TL images alone, referred to TL nuclei model (Figure 2).

Similarly, for TL based segmentation model of cells, we utilized a pre-trained AI model to segment cells in Calcein AM-stained images. The resulting segmentation masks, paired with their corresponding TL images were used to train a model capable of segmenting cells directly from TL images alone, referred to TL cell model.

After training, the TL nuclei model was then applied to segment nuclei (labeled as Nuclei), and the TL cell model was used to segment cells (labeled as Cells). Additionally, a DAPI-based nuclei model was used to segment nuclei as ground truth (labeled as Nuclei_GT). A user-defined target was created to identify overlapping regions between Nuclei and Nuclei_GT, labeled as Overlapping. These labels were used to calculate performance metrics: Nuclei and Nuclei_GT for detection rate, and Nuclei and Overlapping for false positive rate. Finally, Nuclei counts were used to generate the dose-response curve.

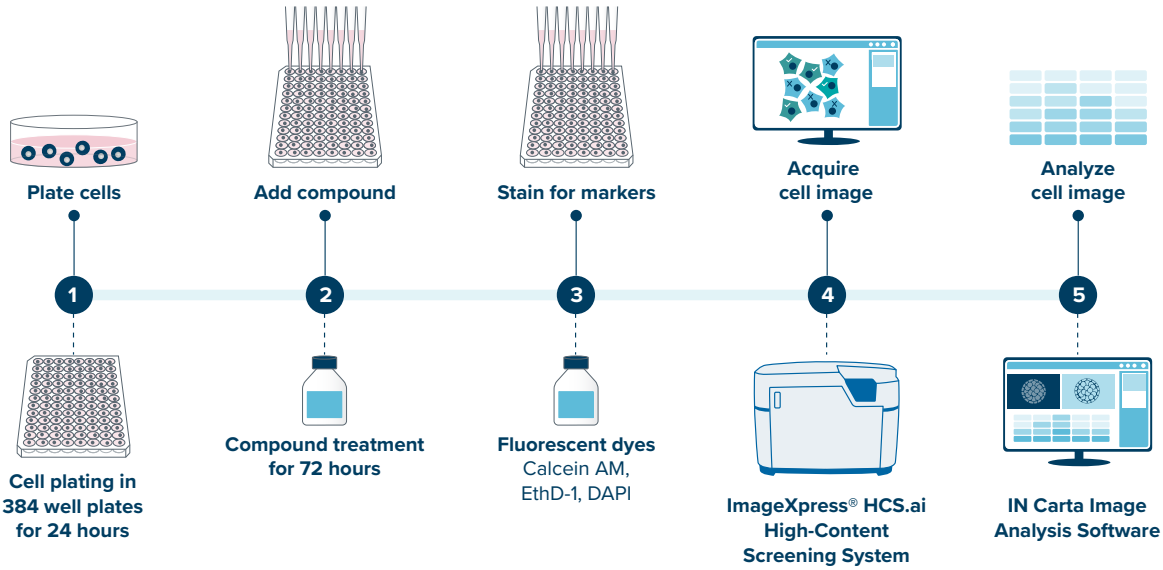


Figure 1. Cell culture and treatment were followed by imaging acquisition, with the resulting datasets analyzed using IN Carta® AI Image Analysis Software.

 |

Results

SINAP allows cross-channel supervision and customizable AI model

Transmitted light (TL) microscopy images present significant challenges for segmentation, a process of outlining regions of interest such as cells or nuclei. The difficulties mainly stem from the low contrast and complex image features inherent in this method, which captures only the light that passes through a sample. To overcome these challenges, deep learning (DL) methods—particularly Convolutional Neural Networks (CNNs)—have become a popular approach. However, even DL methods require careful optimization to perform well on transmitted light images and require curated training data to be effective.

Manually creating ground truth for TL image segmentation is difficult and inconsistent due to its inherent properties as described above. To address this hurdle, we used SINAP module in IN Carta Image Analysis software allowing us to customize the AI model via cross-channel supervision, a common approach in computer vision, which is building training masks from one channel while training a model on another channel1 (Figure 2). We began with U2OS cell line. To train an AI model for segmenting nuclei in TL images, we first utilized a pre-trained model to generate masks from the DAPI channel. These masks were then paired with the corresponding TL images to form the training dataset (Figure 2, Figure 3A). A total of 40 mask-image pair sets were created to retrain a base model. Then a similar approach with the help of Calcein AM channel was implemented to train an AI model that segments cells directly on TL image (Figure2, Figure 3B).

Figure 4 illustrates the results from post-trained models with spatial alignment between the TL nuclei mask and the DAPI staining (Figure 4B), as well as the alignment between the TL cell mask and the Calcein AM staining (Figure 4C), confirming accurate segmentation for both nuclei and cell. Figure 4E presents a comparison of nuclei and cell counts across individual wells. By comparing the TL nuclei count to the DAPI-stained nuclei (serving as ground truth), an overall counting accuracy of 97% with 0.9% standard deviation was achieved.

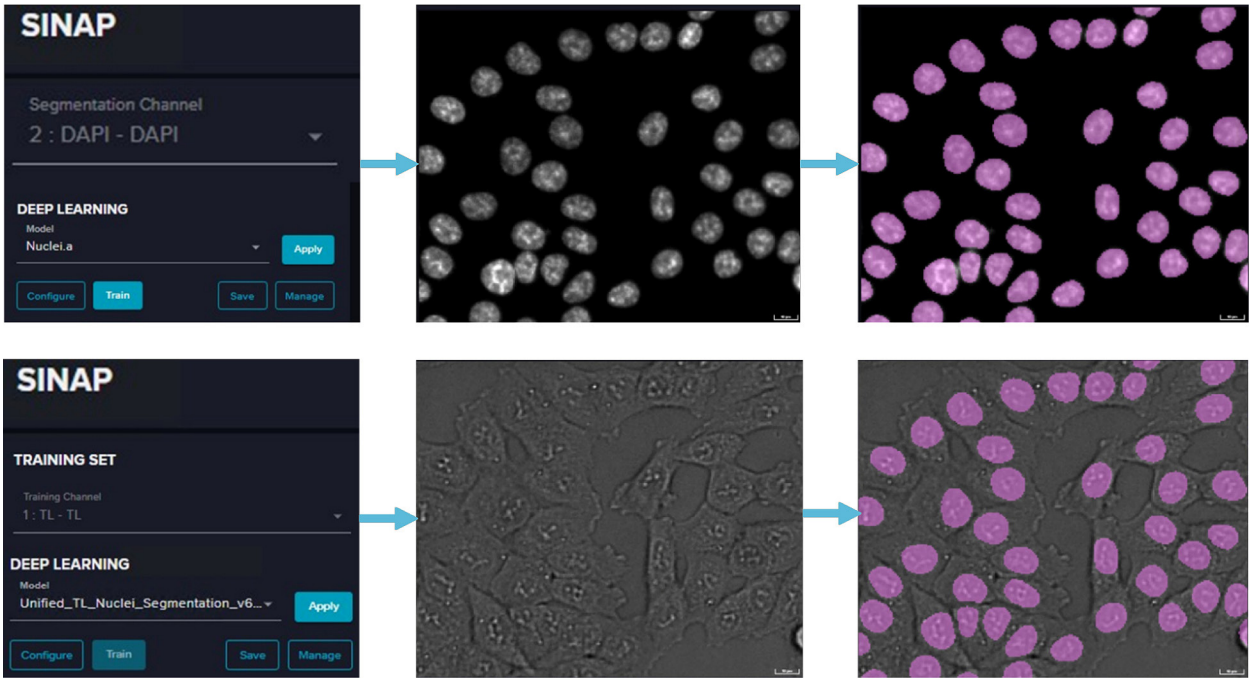


Figure 2. Cross-channel supervision workflow: a DAPI channel was used in Segmentation Channel, and a pretrained Nuclei.a model was implemented to segment nuclei mask. Then a TL channel was used in Training Channel which pairs the nuclei mask with the corresponding TL image to generate the training set. In the last step, the training set was adopted to retrain a base model.

 |

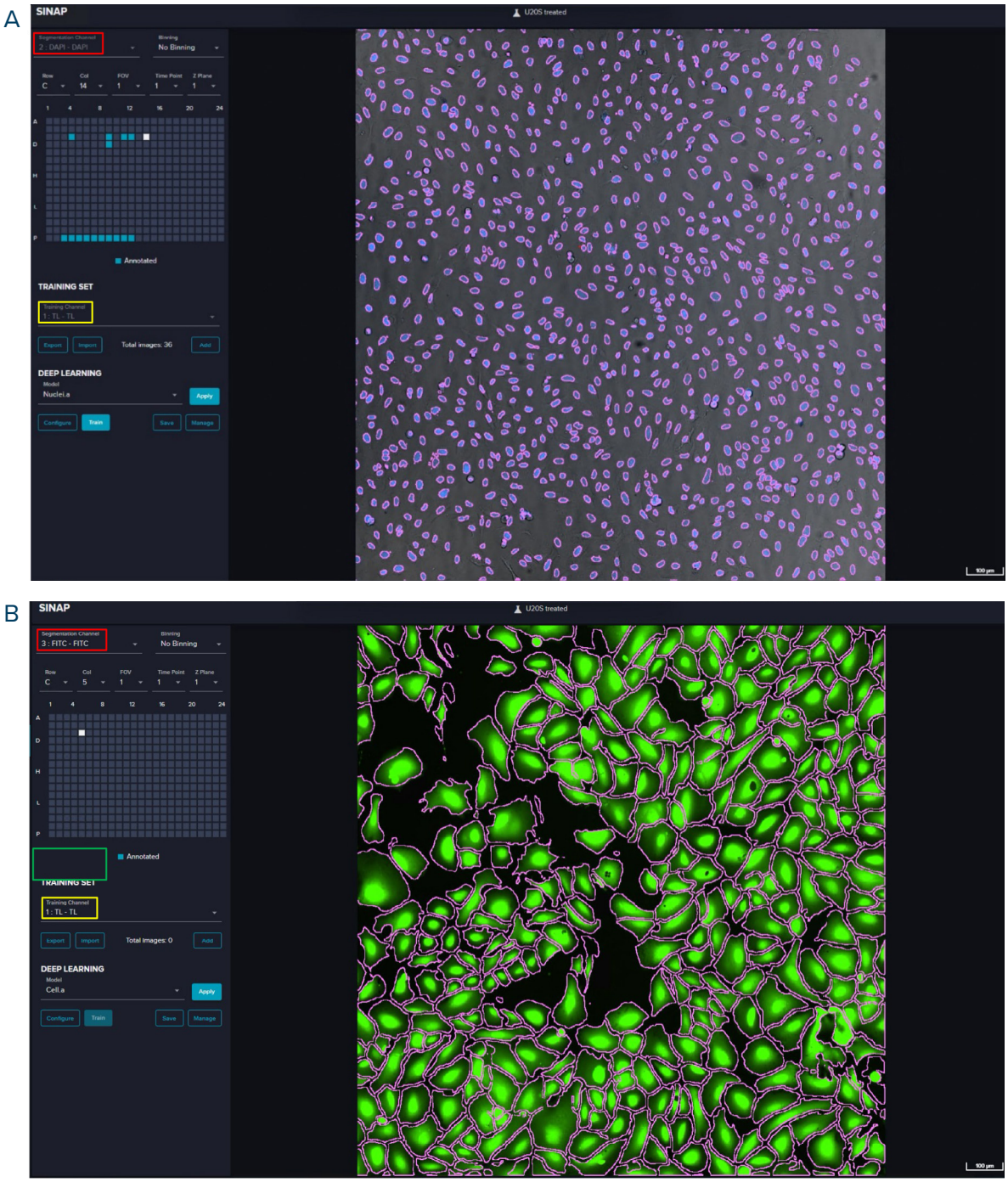


Figure 3. Training interface showing channels selection and segmentation of pretrained models. A. SINAP training interface using DAPI as segmentation channel (red box) and TL as training channel (yellow box). The magenta outlines stand for the nuclei mask generated by pretrained Nuclei.a model. B. SINAP training interface using FITC (Calcein AM) as segmentation channel (red box) as TL as training channel (yellow box). The magenta outlines stand for cell mask generated by pretrained Cell.a model. A U2OS cell line was used in this case.

 |

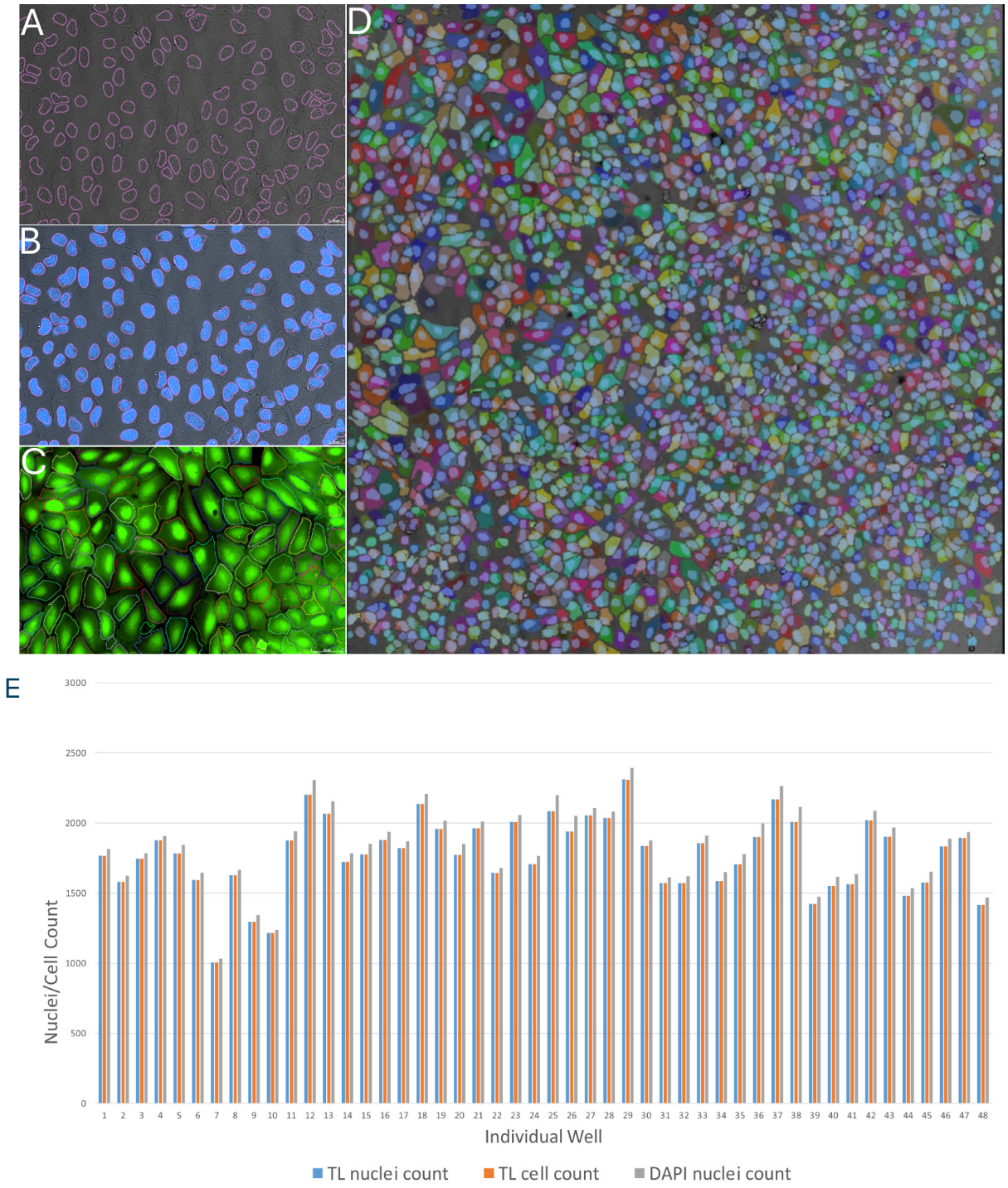


Figure 4. The segmentation results from post-trained TL nuclei model and post-trained TL cell model and the nuclei/cell counts comparison. A. TL nuclei segmentation mask (pink outline) overlayed with TL image. B. TL nuclei segmentation mask (pink outline) overlayed with DAPI image. C. TL cell segmentation mask (random color outline) overlayed with Calcein AM image. D. TL cell segmentation mask (random color) overlayed with TL nuclei mask (blue mask) and TL image. E. Cell count comparison for individual wells. A U2OS cell line was used in this case.

 |

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

Training a multi-cell line model is difficult due to significant cellular heterogeneity, limited data availability, and the challenge of generalizing a model across different contexts. Effectively integrating training data from diverse datasets is essential for enhancing a model's generalizability across various cell lines. Here, we leveraged SINAP's EXPORT and IMPORT features to efficiently assemble the training dataset across five cell lines (Figure 5).

There are two approaches to constructing the complete dataset: parallel and sequential. In the parallel approach, training datasets are exported individually from each cell line and then collectively imported to form the final dataset encompassing all five cell lines (Figure 5A). In the sequential approach, the dataset from all current cell lines is exported and then imported into the next cell line, progressively building the complete dataset (Figure 5B). While the parallel approach can reduce storage requirements for large-sized training datasets, the sequential approach helps the overhead of loading multiple datasets when dealing with many cell lines.

SINAP's User-Defined Target feature enables measurement of the false positive rate

A false positive rate is an important metric to measure a model's performance, which is the proportion of actual negative cases incorrectly classified as positive by a model. To achieve this, we first segmented nuclei from the TL channel and labeled them as Nuclei. A ground truth mask was then generated from the DAPI channel and labeled as Nuclei_GT (Figure 6A). Using SINAP's User-Defined Target feature, which supports customizable binary operations such as AND, OR, NOT, and XOR, we created a mask by applying a binary AND operation between Nuclei and Nuclei_GT and labeled as Overlapping (Figure 6B). Last, we built a target linking by setting Nuclei as Parent and Overlapping as Child and all Nuclei that has zero Overlapping child count are counted as false positive. An example of Overlapping mask generation is shown in Figure 6C–6F.

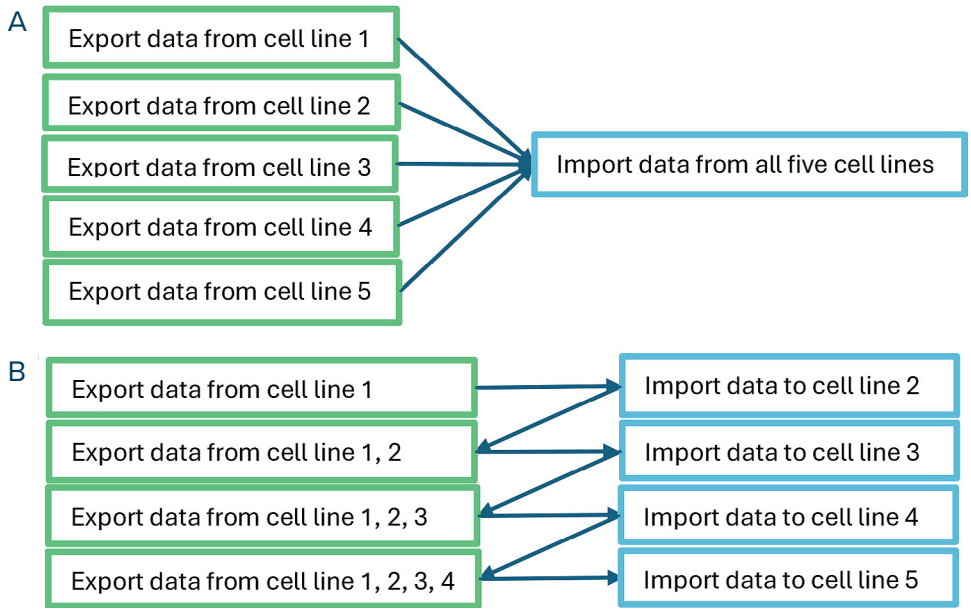


Figure 5. A. Workflow of export datasets parallelly. B. Workflow of export datasets sequentially.

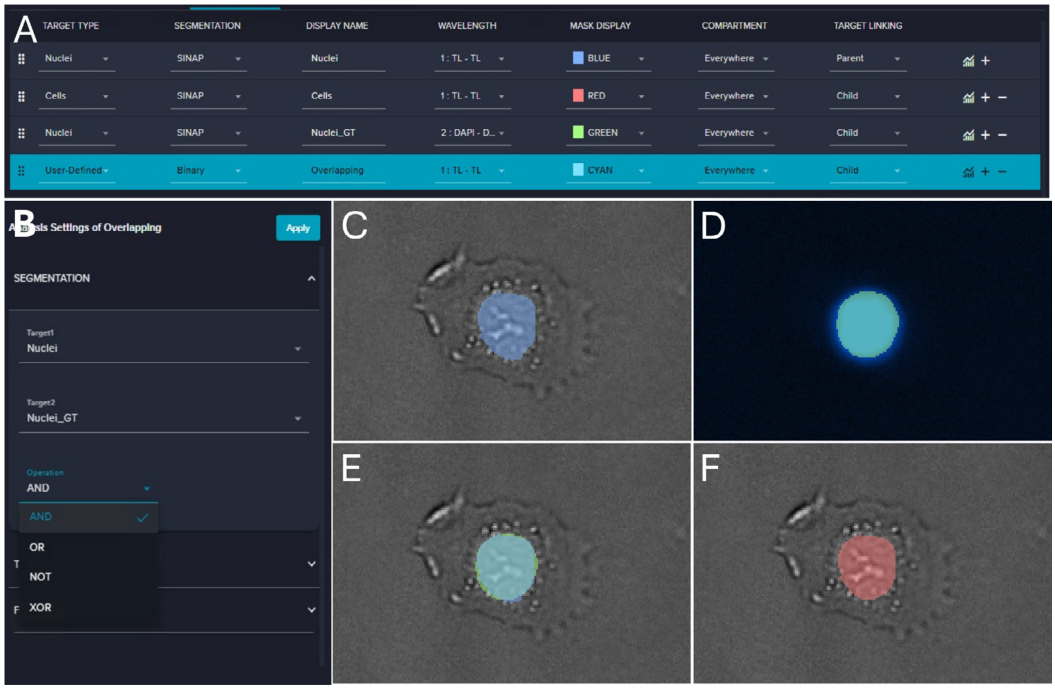


Figure 6. A. Screenshot of the IN Carta analysis workflow. B. Screenshot of setting User-Defined target. C. Overlapping of Nuclei(blue) mask and TL image. D. Overlapping of Nuclei_GT(Green) and DAPI image. E. Overlapping of Nuclei(blue), Nuclei_GT(Green) and TL image. F. Overlapping of Overlapping(Red) and TL image.

Unified TL model detects nuclei in sync with DAPI signals

To assess the unified model's performance, we computed detection and false positive rates across cell lines. Most showed detection rates above 90%, except HEK cells where the rate was ~86%. Notably, for the unified model, U2OS dataset showed ~96% accuracy, which was slightly below the U2OS-specific model (97%) likely due to the influence of additional data from other cell lines (Figure 7A).

The false positive rate is below 2% for most cell lines, with exception of MCF-7, which was ~7%. Further investigation revealed that MCF-7 cells tend to form clusters, leading to poor DAPI staining at cluster centers. This impaired DAPI-based nuclei detection and contributed to the higher false positive rate observed with MCF-7 (Figure 7B, 7C, 7D).

We applied the method to measure effects of anti-cancer compounds on cell number. Treatment with compounds resulted in decreased cell number due to inhibition proliferation and cell death. We generated dose-response curves using TL- and DAPI-derived cell counts, which showed consistent trends (Figure 7E, 7F). The results showed strong correlation and comparable IC_{50} values, confirming the accuracy and reliability of the label-free approach. Here, IC_{50} stands for Inhibitory Concentration 50, which is the concentration of an inhibitor required to reduce a specific biological process or activity by 50%.

The method allowed detection of both live and dead cells, the ones that had intact nuclei.

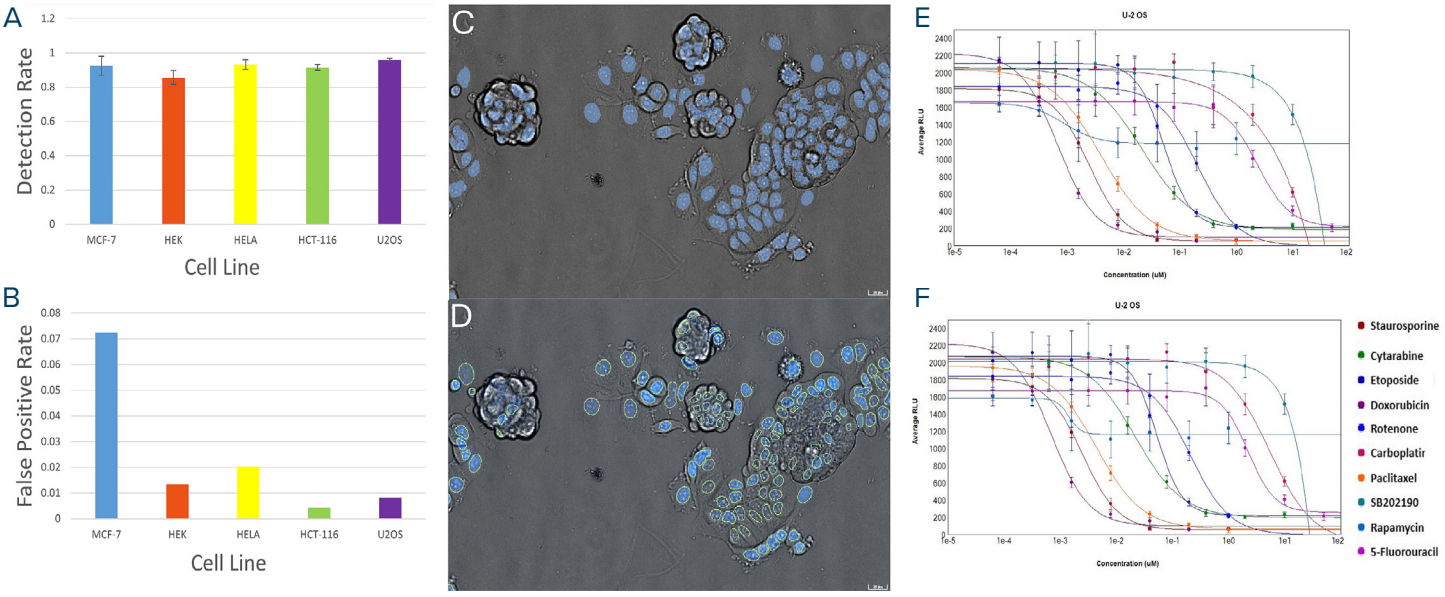


Figure 7. A. The detection rate of all cell lines. B. The false positive rate of all cell lines. C. Overlapping of TL nuclei mask (blue) and TL image of MCF-7 cells. D. Overlapping of DAPI nuclei mask (green outline), DAPI image and TL image of MCF-7 cells. E. Dose-response curve of U2OS cells based on TL-derived nuclei counts. F. Dose-response curve of U2OS cells based on DAPI-derived nuclei counts (other cell lines exhibiting similar behaviors are not shown).

Conclusions

The integration of label-free imaging with a customizable multi-cell line AI model presents a transformative advancement in cellular analysis. By eliminating the need for fluorescent or chemical labels, label-free imaging preserves cell viability and enables real-time, non-invasive monitoring of cellular dynamics. When paired with an AI model trained across diverse cell lines in IN Carta Image Analysis Software, this approach achieves high accuracy in nuclei number detection. The customizable nature of the AI model 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.

Reference

1. Gupta, Saurabh, and Jitendra Malik. *Cross Modal Distillation for Supervision Transfer* (CVPR 2016).

Contact Us

Phone: +1.800.635.5577
Web: www.moleculardevices.com
Email: info@moldev.com

Check our website for a current listing of worldwide distributors.

Regional Offices

USA and Canada	+1.800.635.5577	Taiwan/Hong Kong	+886.2.2656.7585
United Kingdom	+44.118.944.8000	Japan	+81.3.6362.9109
Europe*	00800.665.32860	South Korea	+82.2.3471.9531
China	+86.4008203586	India	+1.800.266.5338

**Austria, Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden, Switzerland and United Kingdom*