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TECHNICAL NOTE

Phenologic.AI: Nuclei segmentation on brightfield images using a pre-trained Artificial Intelligence (AI) model.

Key points

- Brightfield images are rich in information but difficult to segment
- Phenologic.AI™ provides robust segmentation of cells on brightfield images
- Ready-to-use pre-trained AI model compatible with different magnifications and cell lines
- Phenologic.AI performs digital phase image reconstruction for cytoplasm segmentation

Introduction

The use of Artificial Intelligence (AI) for analysis of cellular images is very promising, especially for image segmentation tasks that were previously challenging or unfeasible. The segmentation of nuclei on brightfield images is a prime example of such a task. Brightfield images, while being widely accessible, often suffer from low contrast and lack the specific staining that fluorescent images provide.

To create an effective AI-based module for cell segmentation, the neural network needs to be trained with many ground truth images that need to be representative of the actual sample images. However, the segmentation quality may drop if a different cell type or a different magnification has been used and the images look different from experiment to experiment. Many current AI-based methods therefore require users to re-train their models, sometimes even by manual drawing on images, e.g., to mark nuclei or cells, which is time-consuming and cumbersome.

Pre-trained deep-learning image-analysis Phenologic.AI has been trained on a diverse dataset of thousands of images from various cell lines, captured with different objectives. This extensive training has endowed it with a high level of universality, overcoming the need for training by the scientist. Additionally, automated plane selection and dedicated AI-building blocks enhance the ease-of-use of Phenologic.AI (Figure 1).

Here we show results for 16 different cell lines and for two commonly used magnifications (10x and 20x).

Of the 16 cell lines only two - A549 and MCF7 - have been part of the training data set for the AI model, while 14 are "unknown" to the model. Furthermore, the data used here for A549 and MCF7 are from different experiments (cell seeding at a different lab using a different stock) than the training data.

Phenologic.AI also allows to reconstruct digital phase images if two planes are acquired. Examples of two cell lines with different morphologies are shown in Figure 2.

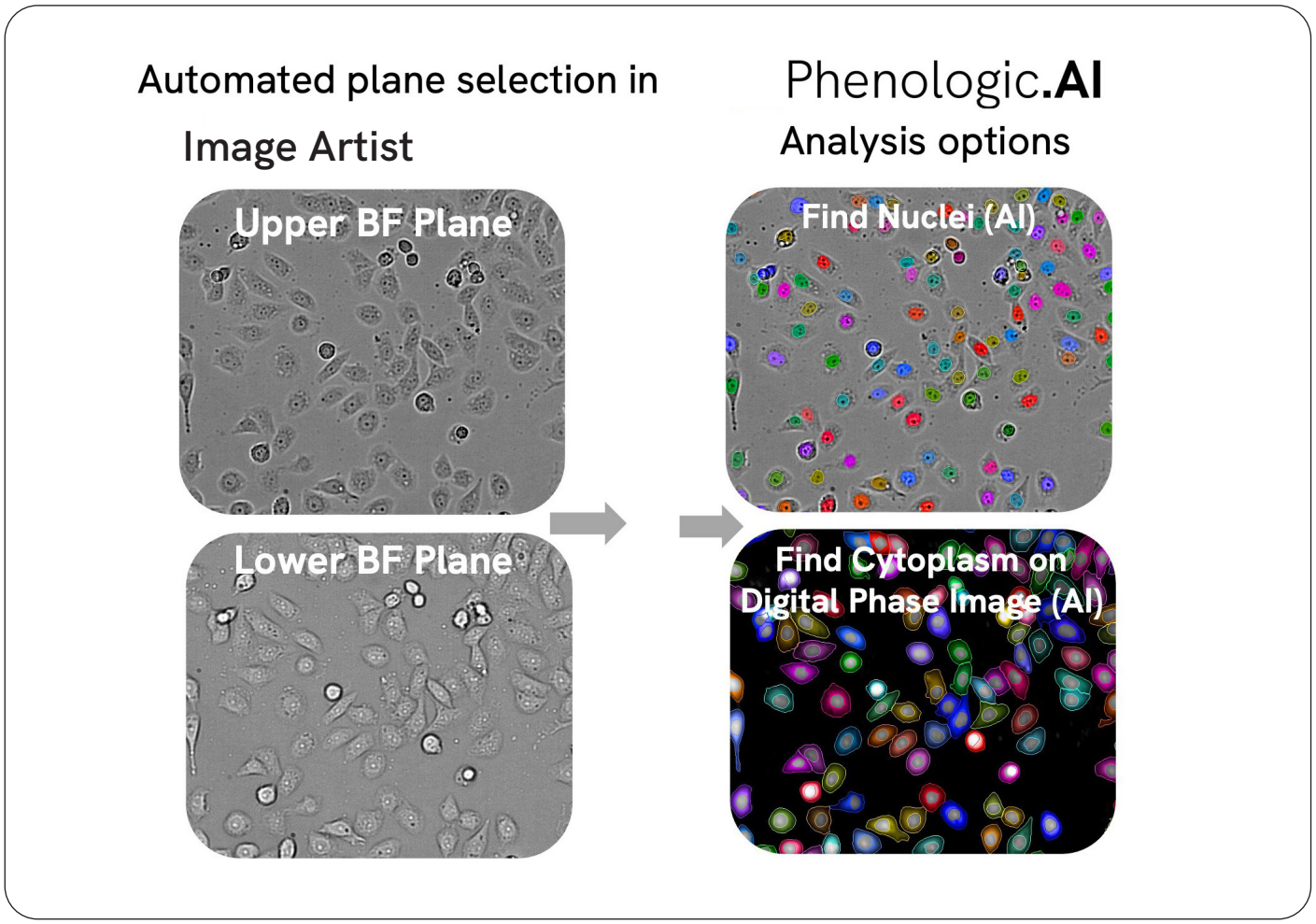

Figure 1: The automated selection of two brightfield planes, along with dedicated building blocks for nuclei segmentation and digital phase image construction for cytoplasm detection, enhances the ease-of-use of Phenologic.AI.

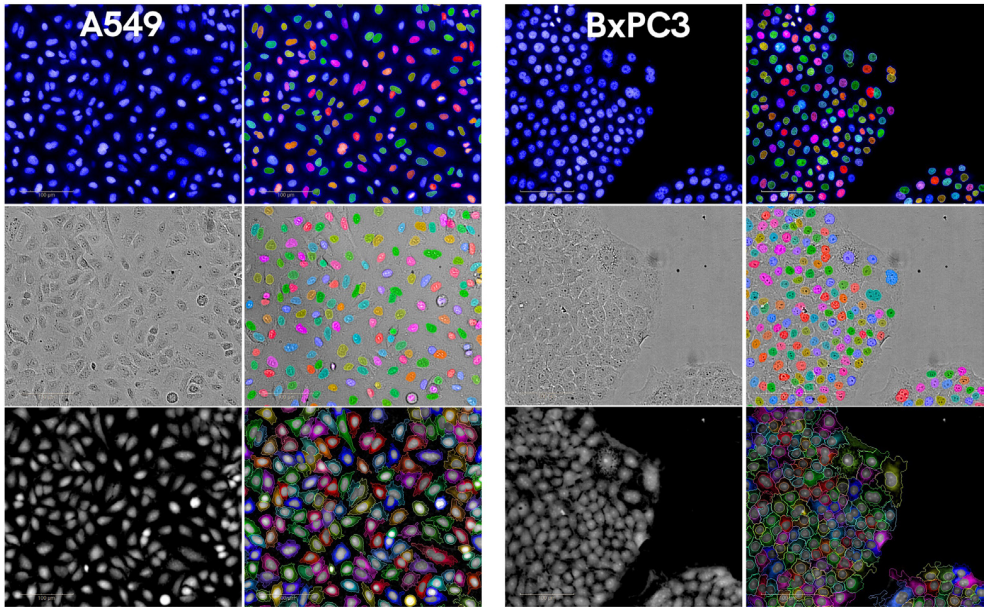

Figure 2: Example images of segmentation results of two cell lines: A549 cells being equally distributed with well separated nuclei, and BxPC3 cells as an example for insular growth pattern and narrower cells. The first row shows the ground truth nuclei segmentation on Hoechst channel, and the second row the AI-based nuclei segmentation based on brightfield images. The last row is an example for a cytoplasm segmentation using an AI-based digital phase contrast image.

The new Phenologic.AI building block is available in Image Artist™ and is shown in Figure 3.

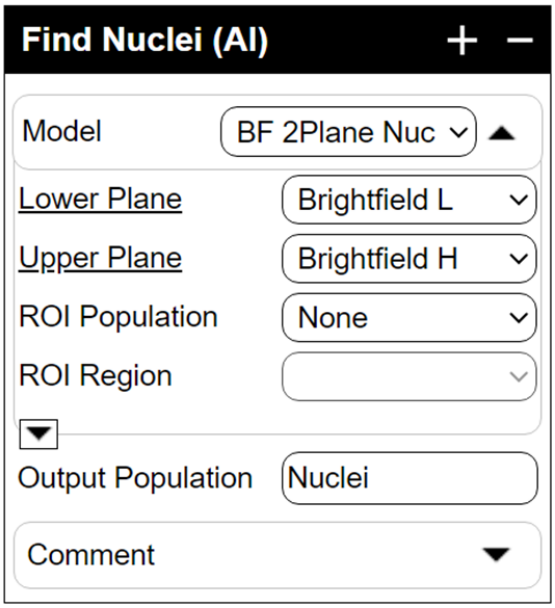

Figure 3: Phenologic.AI Building Block – Find Nuclei (AI) The model allows to switch between 1 plane and 2 plane model for nuclei detection. Upper and lower plane need to be acquired in separate channels (not as stack).

In addition to brightfield, PhenoVue™ Hoechst 33342 images were acquired and analyzed using the "gold standard" Find Nuclei building block of Image Artist software as a reference. This segmentation was used as the ground truth and false positive detection by Phenologic.AI identified by the lack of overlap between both segmentations. Figure 4 shows the results of the detection rate (ratio of Phenologic. AI detected nuclei / ground truth nuclei) and the percentage of false positive nuclei.

For most cell lines and objectives, detection rates above 0.9 were achieved, with less than 4% false positives.

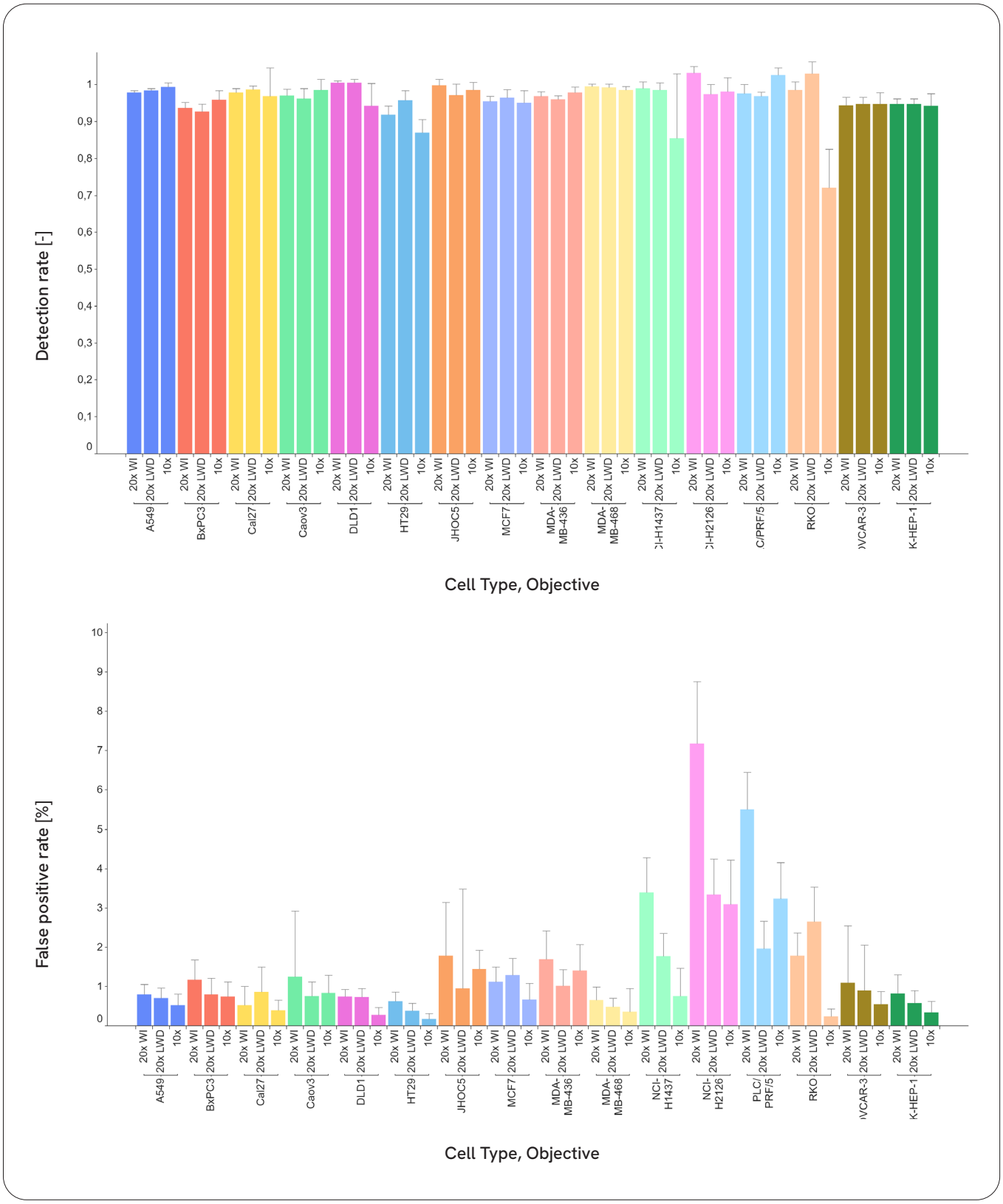

Figure 4: The detection rate of the brightfield AI model on 20xWI (water immersion), 20xLWD (long working distance) and 10x objective images was above 0.9 for all cell lines. **(A)** The ratio of the number of detected nuclei in brightfield to those in fluorescent channel yielded values above 0.9 for both 10x and 20x objectives across most cell lines. **(B)** For most cell lines, the percentage of false positive nuclei was below 4%. Error bars indicate standard deviation, n ≥ 93 wells.

Conclusion

Here, we have shown that the pre-trained AI model was directly applicable to unknown cell lines. For most of the cell lines and objectives, the model yielded ratios of AI-detected nuclei to ground truth nuclei well above 0.9 and false positive rates below 4%. This underscores the universality of the model's usage, alleviating the time and computational demands of model training. This opens up new application workflows for both live and fixed cell applications. Cells can easily be segmented into nuclei and cytoplasm (in combination with the AI-generated DPC channel), allowing not only the use of the cell area as a surrogate marker for cell proliferation but also the counting of individual cells and relating this to cell morphology parameters if needed. Since no fluorescent dye is needed for nuclei or cytoplasm detection, this approach is extremely gentle to cells, allowing short imaging intervals with minimal disturbance. For fixed cell applications, this allows the omission of widely used Hoechst or DAPI staining, enabling the use of another target-specific stain, such as PhenoVue Fluor 405 – Phalloidin.

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