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

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## 1 Introduction

- AI-based image analysis has gained huge interest due to its promise to enable image analysis tasks that were not possible before, e.g., segmentation of nuclei on brightfield images and, also reduces end-user bias in threshold segmentation.
- Brightfield images are rich in information but clearly lack the contrast and specific staining present in fluorescent images, making segmentation a more difficult or very challenging task for most image analysis software solutions.
- Here, we present data showing that a pre-trained model can segment nuclei on brightfield images with very good precision. The pre-training was done on several thousands of images from different immortalized cell lines acquired with different objective lenses to derive a universal deep learning model.
- An advantage of this method is that there is no need to invest time and computational power for AI training process and validation before analyzing data.
- AI based nuclei segmentation alleviates the need of a fluorescent dye for image segmentation, making it an ideal model for *most* live cell applications. Using brightfield images for segmentation, short imaging intervals are possible making it an ideal tool for *most* cell tracking applications.
- The same data can be used to generate an AI-based digital phase image less prone to image artifacts compared to conventional digital phase constructions. Thereby, the cells can be segmented into nuclei, cytoplasm and whole cell.

## 2 Workflow

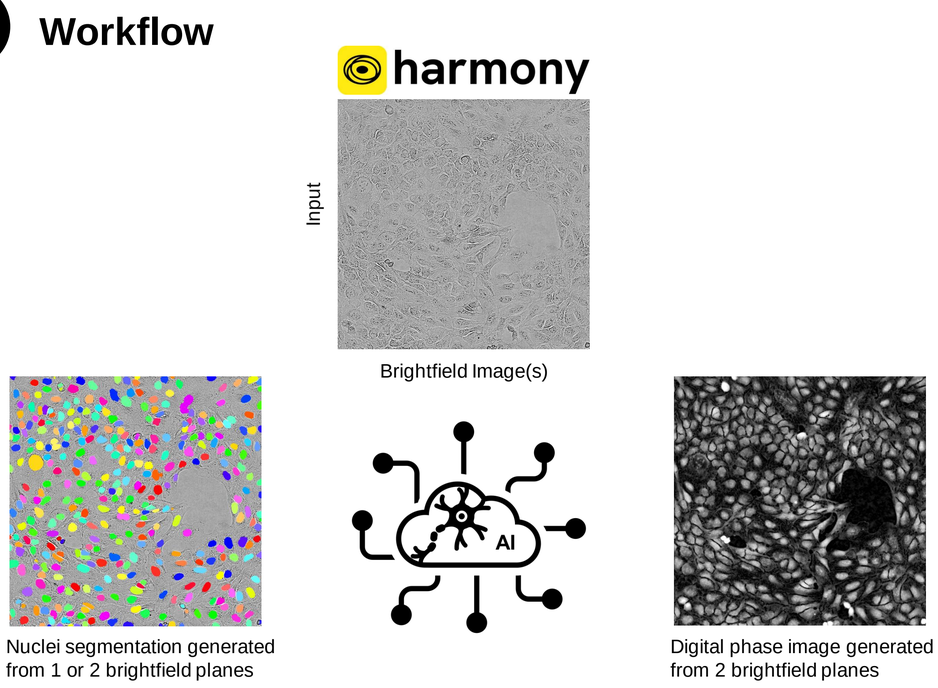



**Figure 1**: Overview of AI based workflow

Nuclei can be segmented on data from either one or two brightfield planes. For AI based digital phase image generation two images acquired in different z-heights are required.

## 3 AI model is compatible across different magnification objective lenses and cell types

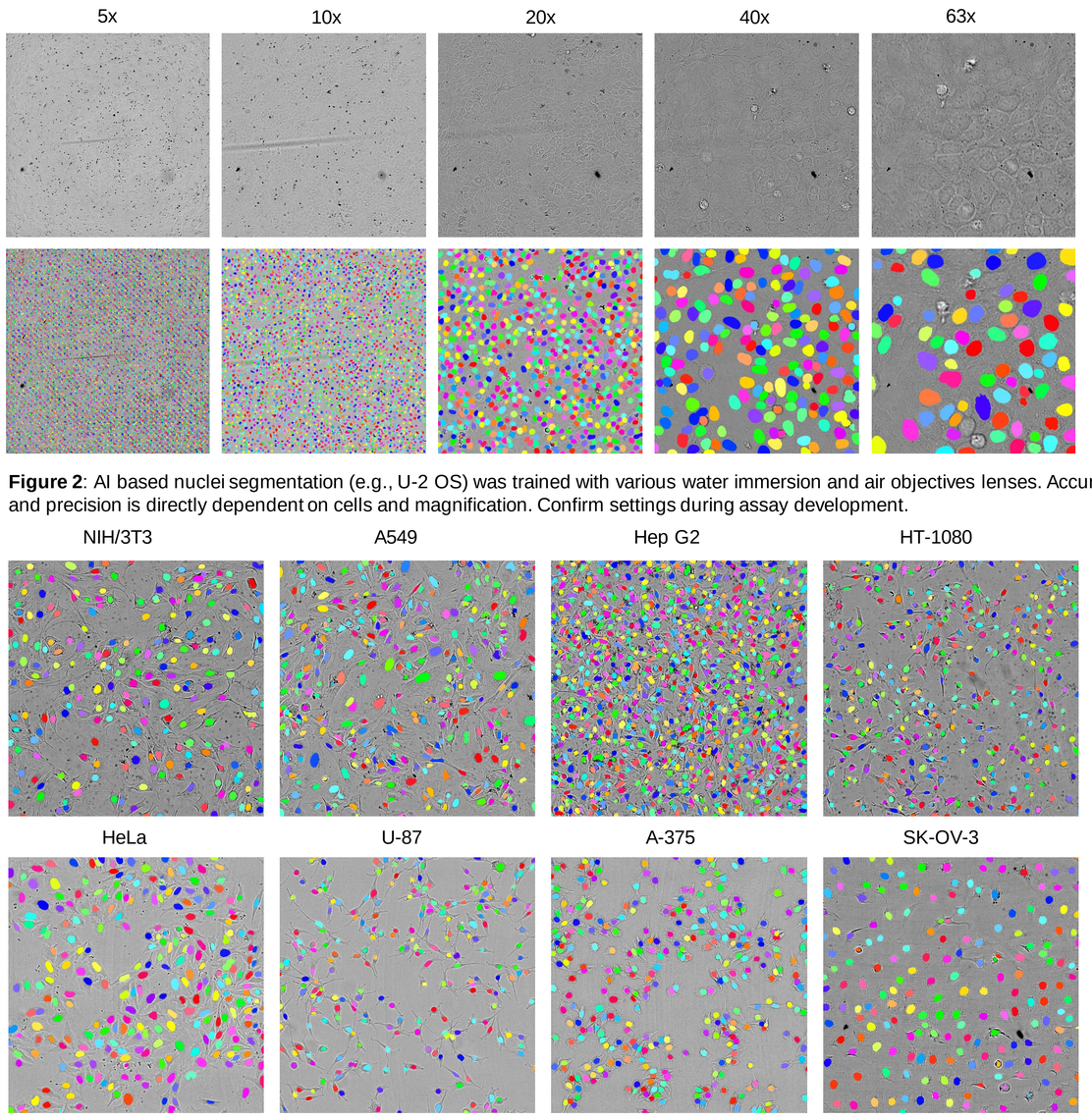



**Figure 2**: AI based nuclei segmentation (e.g., U-2 OS) was trained with various water immersion and air objectives lenses. Accuracy and precision is directly dependent on cells and magnification. Confirm settings during assay development.

**Figure 3**: Pre-trained AI based nuclei segmentation is conducive with many different immortalized cell lines.

## 4 AI enables label-free segmentation of nuclei and cytoplasm

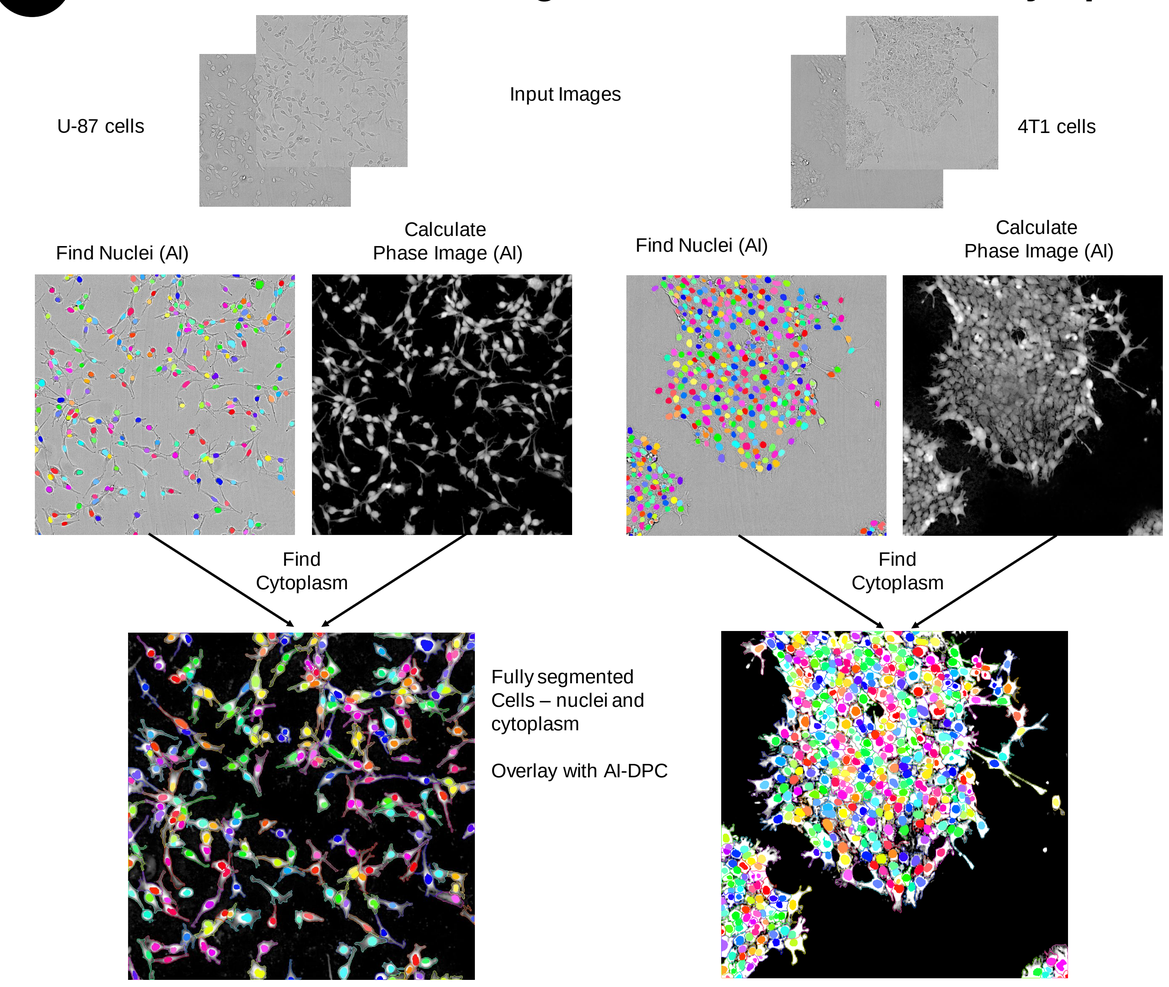
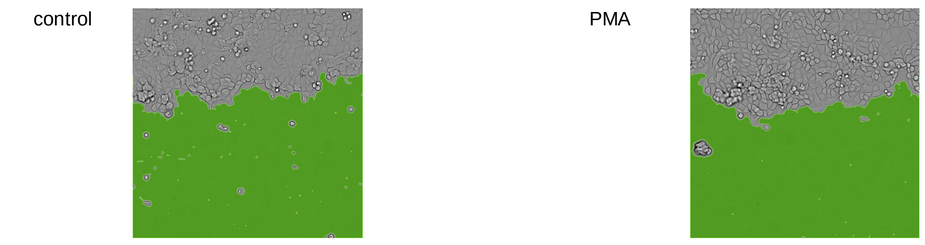



**Figure 4**: Two brightfield images acquired in different z-heights, nuclei are segmented, then used to generate digital phase images. This enables further segmentation into nucleus and cytoplasm. Shown are examples from U-87 (left) and 4T1 (right) cells. Images were acquired using a 20x objective lens.

## 5 AI model is compatible with living cells and enables live cell tracking and single cell readouts

- Live cell wound assay (scratch assay)
- Cell model: MCF-7 cells
- Treatment: 1µM PMA
- Time series: 24h 16min - 4min interval (365 timepoints)



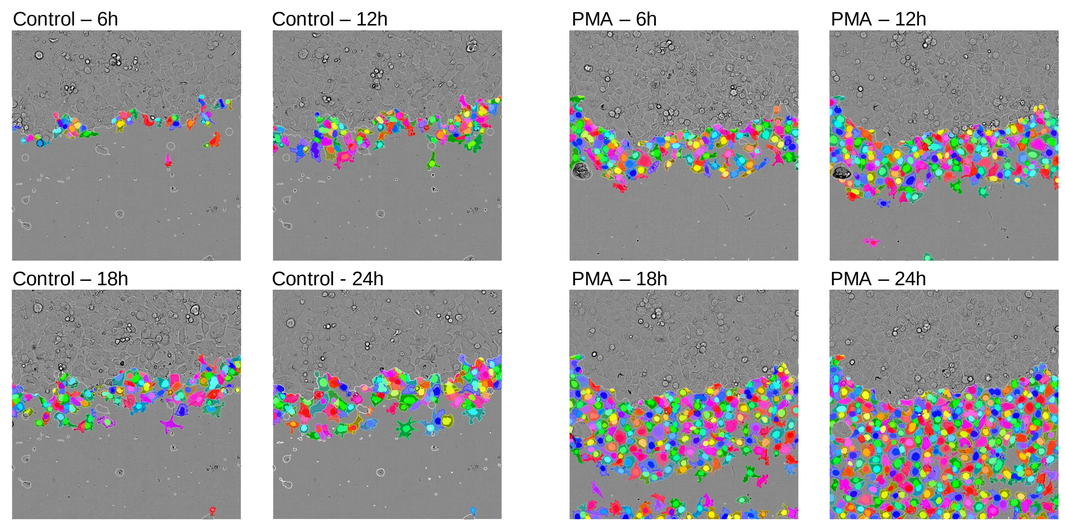




**Figure 5**: Time dependent experimental design at 6, 12, 18, 24 hours after disrupting the MCF-7 monolayer. Control conditions (left) and PMA treated (right) images were captured and segmented. Observation shows an increase in migration with PMA.

Following the brightfield segmentation process, the number of cells inside the wound area were calculated for each time point As cells were segmented, the percentage of wound area closed per timepoint was calculated revealing the wound area closing by approximately 25% in the untreated vehicle control and 75% in the PMA treated condition (figure 6). Due to the gentle nature of brightfield imaging conditions, acquiring images as little as 4-minute intervals is possible to allow precise tracking of individual cells (data not shown).

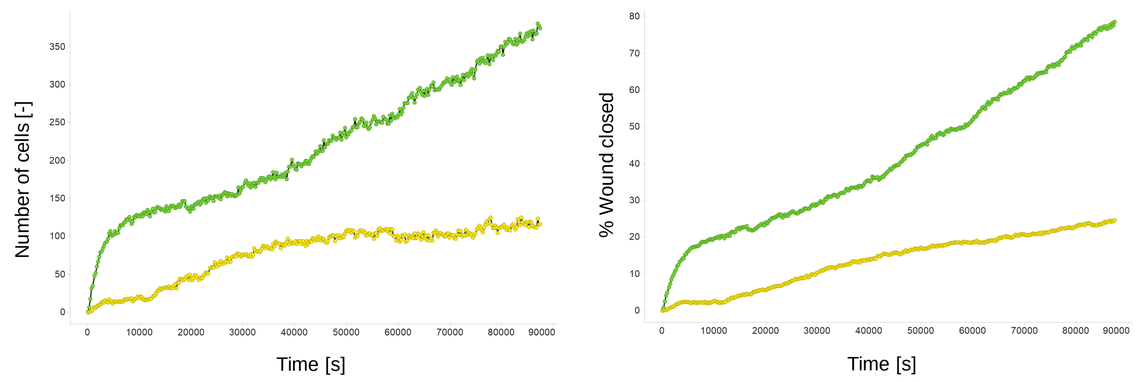



**Figure 6**: MCF-7 cells wound healing closure over time with PMA treated conditions. Yellow ( ) represents untreated control condition and green ( ) represents PMA treated conditions. Left shows number of cells (y-axis) in each treatment versus time (x-axis). Right shows percent wound closure area (y-axis) in treated conditions versus time (axis).

## 6 Conclusions

- AI model is integrated into Harmony® software of Opera Phenix® Plus and Operetta® CLS™ – no training and parameter tuning is required for using it on *most* cell types.
- Demonstrated the AI model can rapidly identify and segment nuclei from various cell types from label-free brightfield images.
- AI model is compatible with different objectives/magnifications.
- AI-Phase images enable segmentation into cell, nucleus and the cytoplasm for advanced image analysis processing.
- This approach is ideal for live cell applications even for sensitive cell types using short imaging intervals.
- Brightfield imaging approaches expands multiplexing capabilities in combination with fluorescence channels.