Label-Free Cell Nuclei Analysis with AI

Automated analysis of cell populations in microwell plates, without the need for fluorescent labeling, offers researchers a number of significant benefits. For the process to be reliable, however, it is essential that protocols are both robust and easy to use. Methods based on artificial intelligence (AI) are ideally suited to address this challenge. Using the right software, AI-based image analysis requires minimal human input and a short training phase. Here, we demonstrate as an example application for the new self-learning microscopy approach how AI can reliably detect and analyze cell nuclei from unstained brightfield images with an accuracy that exceeds fluorescence-based methods.

Label-free analysis of cell nuclei benefits cellular research in different ways. It simplifies sample preparation, enables faster imaging, saves fluorescence channels for other purposes and reduces phototoxicity. However, without stains or fluorescent labels, image contrast is often low. Moreover, when looking at live cells in microwell plates, challenges such as shading, condensation and particles in the medium add further complications to the analysis (fig. 1). Automated image analysis is often time-consuming due to the complex nature of setting parameters for quantification. This can be simplified using AI based on deep learning and convolutional neural networks, which makes applications such as label-free analysis both robust and user-friendly.

To Label or not to Label

To validate the capability to detect and separate cell nuclei reliably, we applied the AI-based 'self-learning microscopy' approach to the brightfield analysis of fixed HeLa cells in a 96 well plate. We captured unstained brightfield images along with fluorescence (GFP) images to generate 'ground truth' data using Olympus' scanR high content screening (HCS) microscope and a 10x UPLSAPO objective.

These pairs of images served as input during the neural network's training phase, which took less than 90 minutes. The network automatically learned to use the brightfield images to generate a probability image that indicates the predicted positions of nuclei. We validated these results by taking randomly selected nuclei from a large validation data set and comparing both the fluorescence and AI output to the brightfield image (fig. 2).

With these encouraging results we went one step further and looked at places where the two methods produced a different result. For example, when studying the area distribution of objects in the fluorescence images, we found three times more unusually large objects compared to the AI result (2% vs. 0.6%). The software enabled us to take a closer look at these large objects. When comparing them to the brightfield images, we noticed that in many cases the object consisted of two nuclei that could not be separated in the fluorescence image. In many cases, the AI approach correctly identified the object as two separate nuclei, showing that AI outperformed the fluorescence-based method, leading to more accurate quantification (fig. 3).

AI, that's Why

The use of AI-based self-learning microscopy brings an invaluable combination of robustness and ease of use to many high-throughput image analysis applications. Here, we have demonstrated that the deep learning approach provided by Olympus' scanR HCS software detects cell nuclei from brightfield images more reliably than fluorescence-based methods. The software achieved this after only a short, automated training stage, and the learned protocol can be applied to new samples easily.

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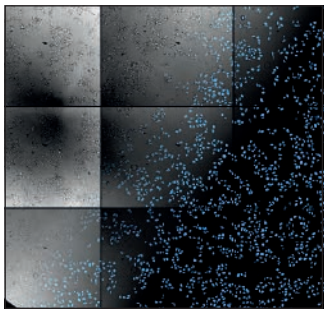

Fig. 1: Brightfield image of a microwell showing artefacts (scratches, condensation, meniscus effect) that can make automated quantification unreliable, and AI prediction overlay (blue).

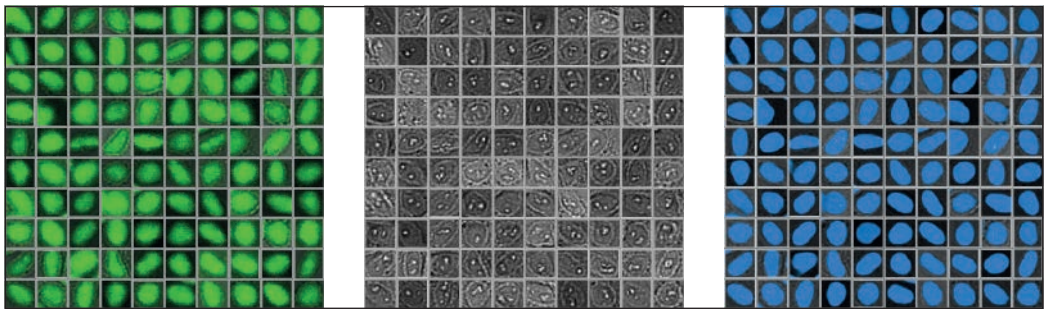

Fig. 2: GFP-labeled nuclei (a), brightfield images (b) and AI prediction from brightfield images (c).

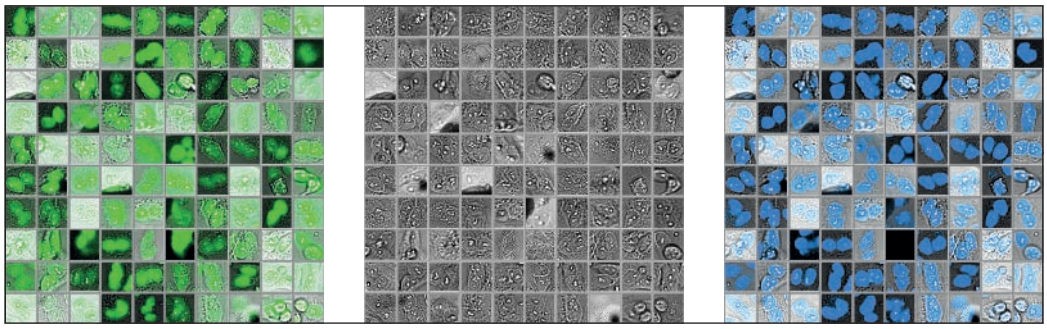

Fig. 3: Sample of large objects in fluorescence (a), brightfield (b) and AI prediction on brightfield (c).