# ROBUST OPTICAL AUTOFOCUS SYSTEM UTILIZING NEURAL NETWORKS APPLIED TO AUTOMATED MULTIWELL PLATE STORM MICROSCOPY

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**KEY WORDS:** Autofocus, automated, high content microscopy, deep learning, super resolution, STORM, neural network

We present a robust, neural network-based optical autofocus system that can operate over a range of ±100µm with submicron precision, enabling automated imaging with a 1.3 NA objective lens. A robust autofocus system is important for imaging modalities that require long image data acquisition times, including single molecule localisation microscopy (SMLM) techniques, time-lapse imaging and automated microscopy, e.g. for high content analysis, where thermal or mechanical drift can compromise image data. Our low-cost infrared laser-based autofocus uses a convolutional neural network (CNN) architecture. Critically we show that training the CNN model using data acquired over ~ 10 days can accommodate subsequent drifts in alignment, etc., that cause the system to fail for models trained on data from one day. To address the trade-off between precision and range, we utilise an elliptical laser beam that provides different confocal parameters in orthogonal directions. Using a 2-step homing approach, the autofocus can achieve a range of ±100 µm with a precision of ~200 nm. This autofocus was retrofitted to a motorised fluorescence microscope (Olympus IX-81) to implement automated multiwell plate easySTORM [1] and applied to high content super-resolved imaging, including of focal adhesions in melanoma cells and phagocytosis of bacteria.

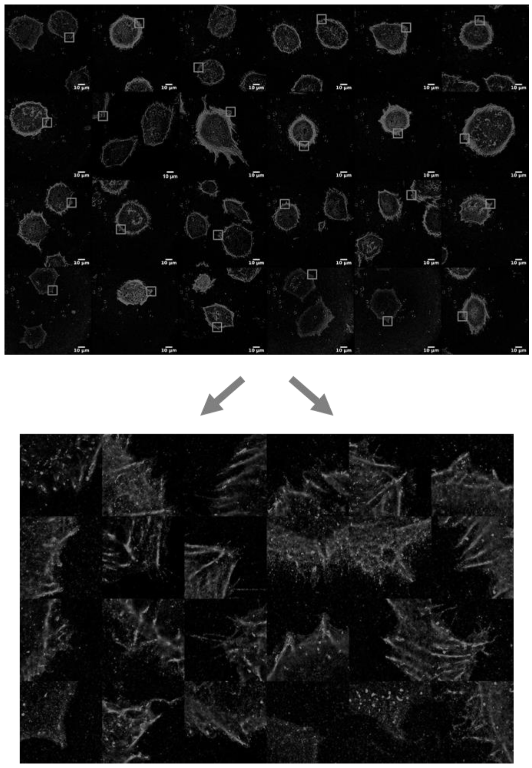

Figure 1. dSTORM images of focal adhesions from automated multiwell plate data set utilising CNN-based optical autofocus system (lower images zoomed in)

[1] Kwakwa et al, “easySTORM: a robust, lower-cost approach to localisation and TIRF microscopy”, J. Biophotonics 9, 948–957, (2016)