Supplemental Document

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Deep learning-based autofocus method enhances image quality in light-sheet fluorescence microscopy: supplement

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Deep Learning Based Autofocus Method Enhances Image Quality in Light-Sheet Fluorescence Microscopy

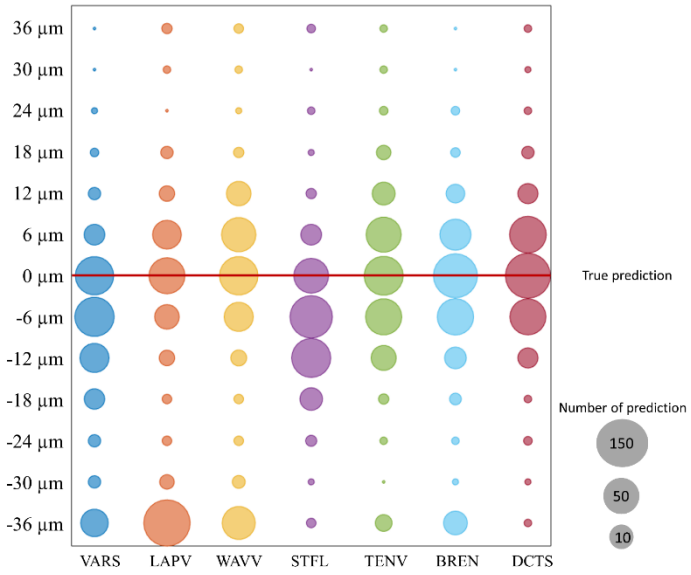

Fig. S1. Comparison between traditional autofocus quality measures. The bubble chart shows the prediction of various methods when a full stack (13 images) is provided as an input. In all the cases (~420) the true prediction is at 0 µm. From left to right, the methods are image variance (VARS), Variance of Laplacian (LAPV), Variance of Wavelet coefficients (WAVV), Steerable filters (STFL), Tenengrad variance (TENV), Brenner's measure (BREN), Shannon entropy of the normalized discrete cosine transform (DCTS). DCTS shows the best performance among the tested methods.

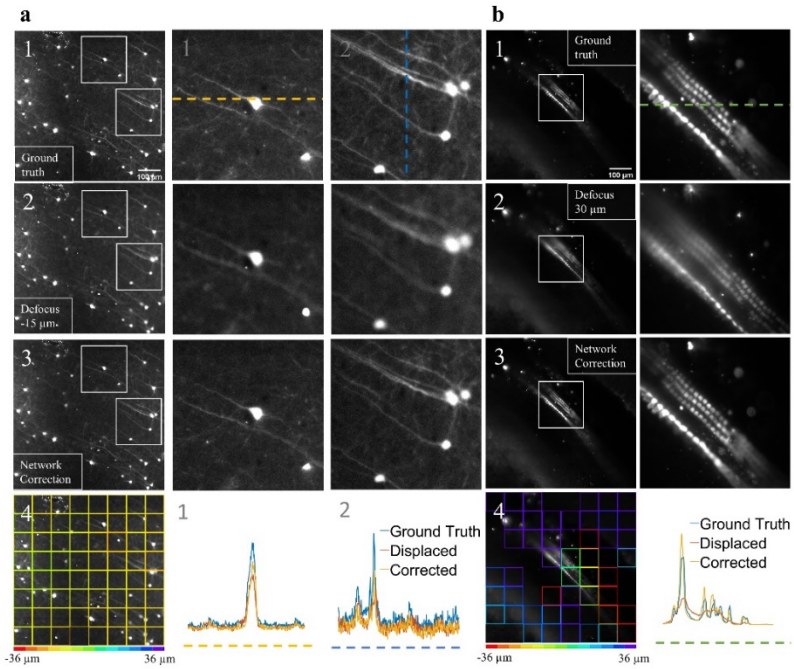

Fig. S2. Real-time perturbation experiments in light-sheet fluorescence microscopy. (**a1 and b1**) The in-focus ($\Delta z = 0$) images of neurons and hair cells, respectively. (**a2 and b2**) Images that show the same field of view as in a1 and b1 after the objective lens was displaced by -15 µm and 30 µm, respectively. (**a3 and b3**) Images of the same field of view after the objective was moved according to the network defocus prediction as shown in **a4** and **b4**. The improved image quality in **a3** and **b3** indicates that the network can estimate the defocus level and adjust the detection focal plane to improve image quality. In **a** and **b**, the white boxes mark the location of the zoom in images, and the line profiles in a4 and b4 represent image intensities along the dashed lines in **a** and **b**.

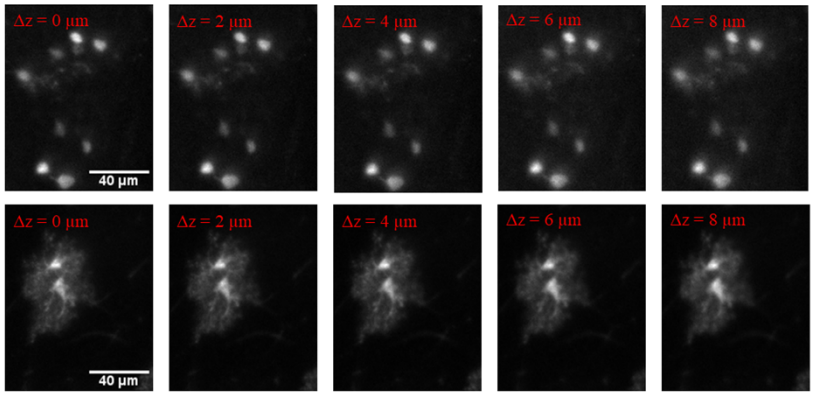

Fig. S3. Changes in image quality over small defocus distances. Representative examples of defocused images with small defocus (Δz) values. Changes in image quality are observed by eye starting from Δz distance of 6 μm.

Table S1. Performance comparison between DNN and DCTS on 3 × 3 tiles with various conditions.

Condition	Absolute distance between predicted and actual position
DNN (with certainty)	3.8976 µm
DNN (without certainty)	4.3048 µm
DCTS (9 tiles and average)	5.1857 µm
DCTS (9 tiles and average with certainty)	4.7599 µm
DCTS (one tile 384 × 384 pixels)	4.1857 µm

Note: all 420 test images are randomly cropped from the test dataset (42 stacks), the size of the test image is 384×384 (pixels), which is exactly 3×3 tiles. In general, DNN performs better when tiles with low certainty are excluded, and DCTS performs better when it is provided the entire 384×384 pixels as input. The certainty values for the DCTS were provided from the DNN.