Fiji Plugin User Manual: Deep-Z Digital Propagation Network

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This plugin performs 3D fluorescence reconstruction from a single 2D fluorescence microscope image, using deep-learning-based digital propagation and time-reversal framework.

For details, refer to our publication ***"Three-dimensional propagation and time-reversal of fluorescence images"***. (Please cite our paper if you find it helpful. Thanks!)

Software setup

This plugin is developed with the **Fiji** version 1.52i, but is also compatible with the latest version. We provide two methods to setup the plugin.

1. **Integrated method (recommended)**
   We provide a Fiji of version 1.52i with Deep-Z Digital Propagation Network plugin integrated. Download and click /Fiji.app/ImageJ-win64.exe to start testing.

2. **Manual installation**

   If you already have a compatible Fiji, you can also manually add our plugin. The procedures are:

   a. Copy all .jar files in /Plugin-jar-pack into [YOUR_FIJI_PATH]/Fiji.app/plugins
   b. Double-click [YOUR_FIJI_PATH]/Fiji.app/ImageJ-win64.exe to launch FIJI application.

Preparing test data

The Plugin currently ***only*** works with fluorescence image of size 1024*1024 pixels. Pad with zeros if you have a smaller image.

Example images are provided in /Data/TestImgs/, there are 5 types of images included, corresponding models are provided in /Data/Models/. (**Note:** Using a model pre-trained with the same objective-lens + sample set generates the optimal result, although testing with a different model usually also works.)

Testing a trained model

1. In Fiji, go to **Process -> Deep-Z Digital Propagation Network** at the bottom, a 'Deep-Z Digital Propagation Network' window will pop out.
2. Select '**Generate Single Height**' or '**Generate Stack**' option to run:
   a. **Generate Single Height**: digitally propagate to only one height and show one layer output
   b. **Generate Stack**: propagate to user defined height range and show an image stack, so that users can scroll back and forth for better visualization.
3. Select **Model Directory** and **Image directory** by clicking 'Browse'. For example, select /Data/Models/Leica_20X0.8_worm_FITC for model directory and then select /TestImgs/Leica_20X0.8_worm_FITC/scan0001_FOV01.png for image. Then click OK.

(NOTE: for model directory select only the folder, not the files inside; for image directory need to select specific image.)

4. If the **Generate Single Height** is selected:
    a. When you click OK, the input image will be shown in a new window, and another 'Choose height' window will pop out. You can move the slider or input a number at the right box to select height (e.g., you can enter (or slide to) "9"). Then click OK.
    b. The input image will be auto-adjusted based at the display for better observation.
    c. The network output (representing the network inference of the sample as if it is captured at a different depth, specified above) will be shown in another window in ~10s (e.g. ~12s in our 3.6 GHz AMD Ryzen 5 1600X Six-Core Processor with 16GB RAM). The 'Choose height' window will pop out again.
    d. Choose another height to propagate the same input. Click OK.
    e. The previous output window will be updated.
    f. If you want to quit the program, click Cancel.
    g. The plugin ends.
5. If the **Generate Stack** is selected:
    a. When you click OK, the input image will be shown in a new window, and another 'Set height range' window will pop out. You can set the start, end propagation height and stack interval for the output stack. Then click OK.
    b. The input image will be adjusted at display for better observation.
    c. The network output will be shown in another window in a few minutes (depending on your machine and stack number you want to generate). The 'Deep-Z Digital Propagation Network' Window will show again. The default model and image directory are from the previous test. You can set new directories by clicking 'Browse'. Or If you want to quit the plugin, click Cancel.

Tips and release note:

1. (Be patient) Since currently the plugin only supports CPU, the testing typically takes ~ 10 s per image per propagation distance (instead of ~0.5 s when GPU is used), examples are:
    a. (12 s per image) 3.6 GHz AMD Ryzen 5 1600X CPU, 16GB RAM, 64-bit Windows
    b. (7 s per image) 3.7 GHz Intel i7-8700K CPU, 32GB RAM, 64-bit Windows Pro
2. Currently only fluorescence images with similar sparsity as the test image can be evaluated, which is partially due to a fixed image normalization parameter implemented in the plugin. Future release will resolve this issue.
3. While the 20X models were trained with -10 µm to 10 µm, the 40X model was trained with -2.7µm to 2.7µm. Propagating outside training range will result in artifacts.
4. Using a PC with less than 8GB of RAM may result in the crash of the software and should be avoided.