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Zhiduo Zhang, Woei Ming Lee, Lexing Xie, Alex Mathews, Xuefei He, "Noise reduction in ultra-low light digital holographic microscopy using neural networks," Proc. SPIE 11202, Biophotonics Australasia 2019, 1120208 (30 December 2019); doi: 10.1117/12.2539548

Event: ANZCOP, 2019, Melbourne, Australia

Noise reduction in ultra-low light digital holographic microscopy using neural networks

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1. ABSTRACT

Live cell imaging is challenging because the difficult balance of maintaining both cell viability and high signal to noise ratio throughout the entire imaging duration. Label free quantitative light microscopy techniques are powerful tools to image the volumetric activities in living cellular and sub-cellular biological systems, however there are minimal ways to identify phototoxicity. In this paper, we investigate the use of neural network to restore quantitative digital hologram micrographs at ultra-low light levels down to 0.06 mW/cm^2 which approximately two orders of magnitude lower than sunlight. By developing an adaptive image restoration method specifically tailored for digital holograms, we demonstrated the 2x improvement in SSIM over existing denoising methods. This demonstration could open up new avenues for high resolution holographic microscopy using deep ultraviolet coherent sources and achieve high-resolution imaging with ultra-low light illumination.

2. METHODS

2.1 Physical Setup

Measurements from Mach-Zehnder interferometer setup is used for our physical experiments. The setup consists of a He-Ne (628.3nm) laser passing through a 40x 0.70 NA microscope objective in the sample arm, interfered with an unmodified reference beam from the same laser. The light level reaching the sample is controlled through NE filters (ND2.3, ND2.6, ND3.0) applied before beam split, to affect both reference and sample arms. We measure the beam power at the sample plane to be [51.63, 0.25, 0.12, 0.06]mW/cm^2, with respect to ND0, ND2.3, ND2.6, ND2.9. This comparable with existing levels in fluorescence microscopy[1]. Our setup results in 0.03um per pixel, with a 1920x1080 pixel field of view. We observe the distance between fringes is around 4 to 5 pixels.

2.2 Neural Network

The U-Net architecture [2] is a common ML network used in image segmentation, identification and improvement, and has recently expanded into the denoising and image enhancement in fluorescence microscopy [1]. In digital holography, the encoding of information in spatial shifts of the fringes suggests that both fine grained information and high-level information in the image are required to effectively discern noise from object. The skip connection, down-sampling and up-sampling design for U-Net is particularly suited to analysing the input at both a localized level and the contextual level. We modify U-Net, originally built for segmentation to be suitable for hologram denoising. The final activation layer is changed to a linear activation, as we seek a non-binary mapping. In our experiments, we use smaller input images, and thus the network depth and size are smaller in comparison. For our regression problem, Negative Pearson's Correlation Coefficient (NPCC) replaces Binary Cross-Entropy as the loss function. Furthermore, we found it to outperform Mean Squared Error (MSE) on our dataset [3].We note that unlike MSE, NPCC can have a constant multiplicative scaling between the compared measurements, however, in off-axis digital holography, the information is carried through the relative difference between neighbouring pixels and is thus immune to this scaling factor. Therefore, it remains a valid measurement of the quantitative volume of the sample.

2.3 Dataset

We test U-Net on its ability to improve low light holograms by observation of microbeads (3um). For each of the three filtering levels we collected useful data from 10 different field of views, along with their corresponding non-filter counterparts. Training the network required 512x512 pixel images, which were randomly cropped from the image captured by the camera (1920x1080), creating approximately 800 training pairs of varying filtering levels. Two previously unseen full-sized images of NE26 and NE30 were used to validate the results of the experiments, each with a random 30 croppings of 512x512.

Biophotonics Australasia 2019, edited by Ewa M. Goldys, Brant C. Gibson, Proc. of SPIE Vol. 11202, 1120208 · © 2019 SPIE · CCC code: 0277-786X/19/$21 · doi: 10.1117/12.2539548

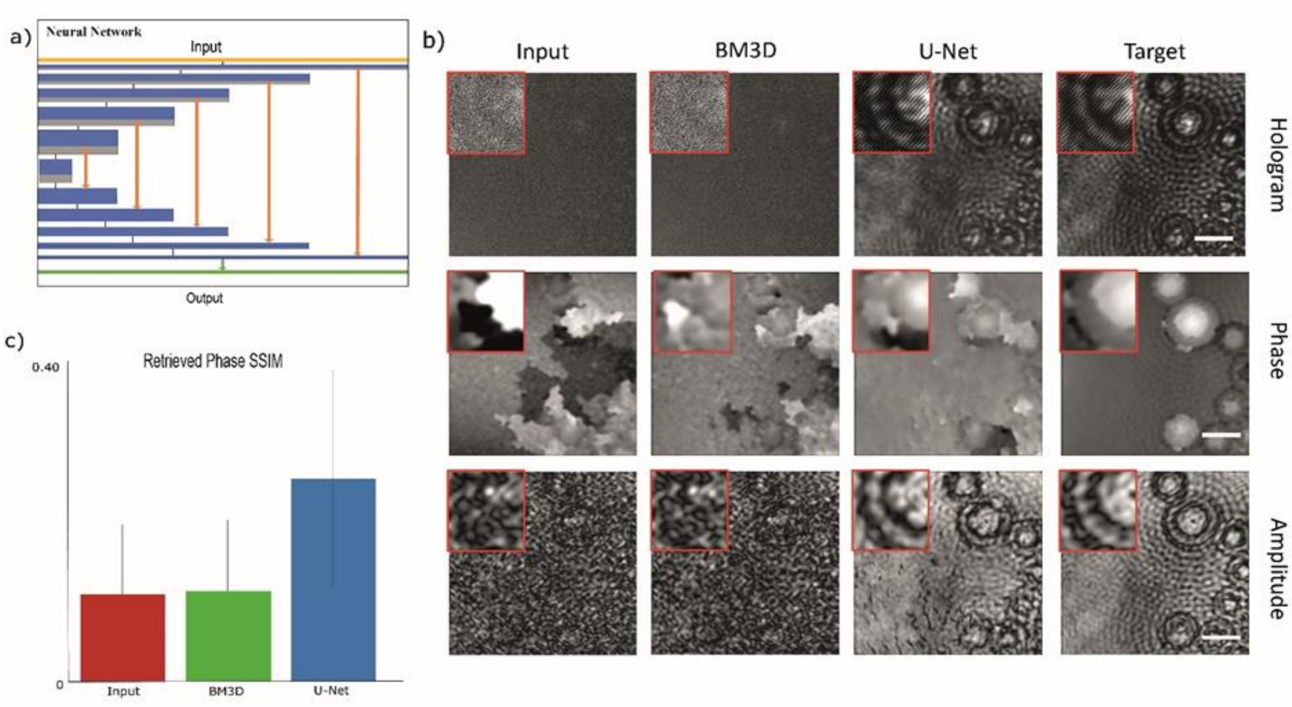

Figure 1. a) Diagram of modified U-Net architecture b) Comparison of our method with BM3D on a test hologram with ND3.0 filtering. The red square indicates a zoomed-in area of the image. c) Comparison of phase quality vs clean target via the SSIM metric between input, BM3D and our method.

3. RESULTS AND CONCLUSION

The modified U-Net is applied to the enhancement of low-light holograms of spherical beads. We compare with the popular denoising method BM3D [4]. In both our method and BM3D, we apply the respective method on the hologram itself. And apply angular spectrum phase reconstruction method [5] to retrieve the phase and amplitude. Our results show that our method offers significant improvements in both phase and amplitude of the reconstructed object when compared with both BM3D and the input. The retrieved phase is on average twice as good via the Structural Similarity Index Metric (SSIM), and the amplitude image demonstrated visibility of the bead structures that were previously invisible in both the input and BM3D. Our method offers a promising path towards low-light digital holography, for both ultraviolet and phototoxicity sensitive experiments. We are continuing our work through the application of the method in a more complex and biologically relevant setting through cell samples.

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