Performance of autofocus capability of deep convolutional neural networks in digital holographic microscopy

Tomi Pitkäaho[a], Aki Manninen,[b] and Thomas J. Naughton[a]

[a] *Department of Computer Science, Maynooth University–National University of Ireland Maynooth, Maynooth, County Kildare, Ireland*

[b] *Biocenter Oulu, University of Oulu, P.O.Box 5000, FI-90014 University of Oulu, Finland*

Abstract: Autofocusing of digital holograms of microscopic objects is a challenging problem. In this paper, an application of a deep learning in autofocusing is described. Its generalisation performance is analyzed.

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1. Introduction

Digital holographic microscopy (DHM) is a label-free, single-shot technique that is well suited for imaging living three dimensional samples [1]. DHM overcomes a problem present in conventional optical microscopes of a shallow depth-of-field, permitting one to reconstruct at different in-focus planes of a volume. Nevertheless, an object of interest is usually in-focus only in one or few depths as a single reconstruction layer still carries a shallow depth-of-field. Different methods to find objects in focus have been proposed. These methods can be based on self-entropy [2], amplitude analysis [3], power spectra [4] or other metrics [5]. Common to all of these single wavelength methods is that they reconstruct a stack of images that the focus metric is applied to. This procedure is applied to each of the holograms. We propose to use deep learning as an autofocusing method. The greatest benefit of the proposed method is that after the training is completed, the in-focus plane can be obtained by using only the single in the hologram plane and without any reconstruction.

2. Digital holographic microscopy

A magnified digital hologram $H_0(x,y) = |R|^2 + |O|^2 + R^*O + RO^*$ can be propagated at any depth z of the reconstruction volume using the Fresnel approximation. From the complex-valued reconstruction, $U(x,y;z)$, the amplitude component is defined as $A(x,y;z) = \{\mathrm{Re}[U(x,y;z)]^2 + \mathrm{Im}[U(x,y;z)]^2\}^{0.5}$.

3. Deep learning

Deep convolutional neural networks that are one form of deep learning have been used successfully in various different visual object recognition and object detection applications [6, 7]. Some of the layers in these networks perform convolution operations on its two-dimensional input.

We chose a convolutional neural network approach to tackle the autofocusing problem still existing in digital holographic microscopy of transparent samples. The architecture of the network is based on the AlexNet architecture that won the Large Scale Visual Recognition Challenge 2012 (Fig. 1) [7].

3.1. Training

In total 494 holograms of semitransparent Madine Darby canine kidney (MDCK) multicellular samples were prepared and captured by using an off-axis Mach Zehnder digital holographic microscope (Lyncée Tec T1000, Lyncée Tec SA, Lausanne, Switzerland) with 660 nm laser source and 40X microscope objective. Each 1024×1024 hologram was preprocessed by removing the zero order and the twin term. Each preprocessed hologram was numerically reconstructed at the middle region of a sample by using a manually chosen reconstruction depth. Each hologram was reconstructed to 20 other depths (±100 mm from the in-focus plane) with the reconstruction step being 10 mm. This process led to 21 classes (labels). The data was processed and augmented by scaling each amplitude reconstruction down to 256×256

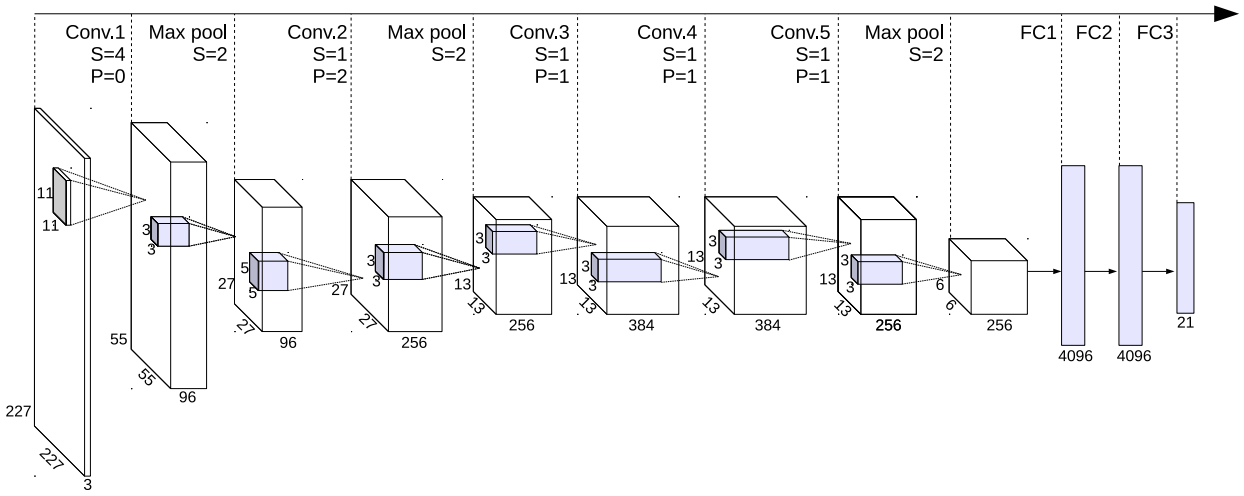

Fig. 1. Network architecture. Conv = convolution, S = amount of stride, P = amount of zero padding, FC = fully connected.

pixel image, that was followed by five 227×227 cropping operations to each corner and the center. Each cropped image was rotated three times (90 degree rotations) and each resulting image was augmented through horizontal mirroring. In addition each 1024×1024 pixel amplitude reconstruction was scaled down to 227×227 pixel image that underwent the same rotation and mirroring augmentation procedures. After data processing and augmentation the total amount of images used in training was 485 856, which was divided to actual training data (90%, 437 271) and validation data (10%, 48 585). The learning rate was set to 0.001. During the training a mean pixel value over the whole training set was subtracted from each training image pixel.

4. Results

The learned filters from the first convolution layer are shown in the Fig. 2. The network was tested with holograms that were not used in training or validation. The testing incorporated holograms of the same type of samples captured with the same setup that were used in training and validation (Fig. 3a). In addition to MDCK samples, a human cell line sample, was prepared and captured with the same hardware (Fig. 3b). Later this sample was fixed and captured by using a different digital holographic microscope with a 20X microscope objective (Fig. 3c). A conclusion from these tests is that the learned network generalises well to different kind of cell samples captured with the same setup, however if the magnification and illumination conditions are altered the network fails. The different filter sizes at each of the 5 convolutional layers may have a big effect on generalisation, and should be studied more carefully to ensure scale invariance.

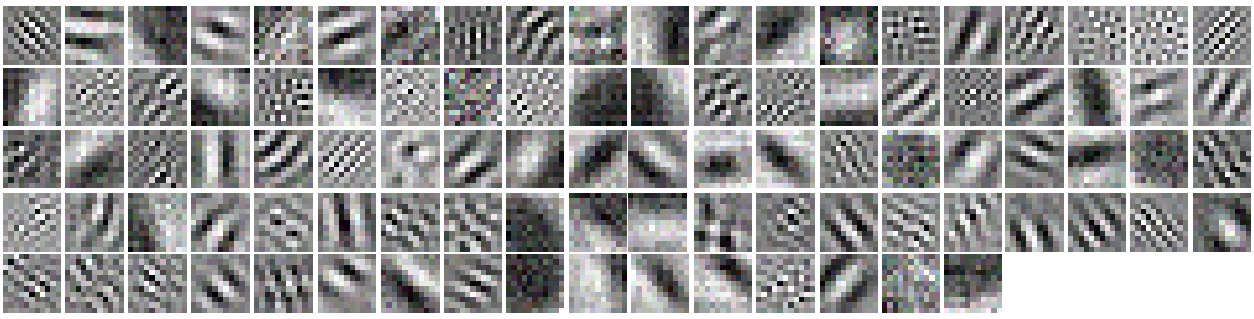

Fig. 2. The 96 learned 11×11 filters from the first convolution layer.

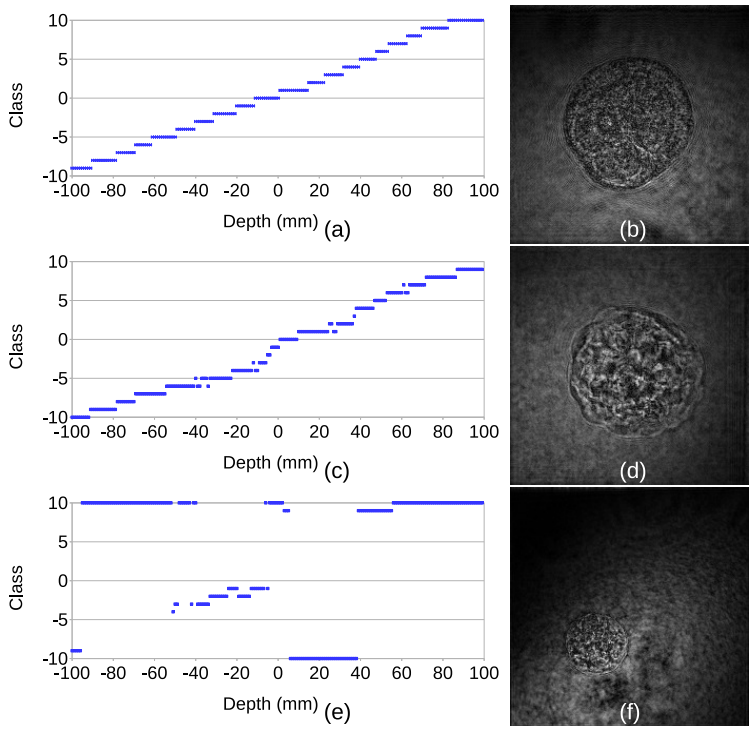

Fig. 3. Test results. Each of the holograms was reconstructed from the center region ±100 mm with steps of 10 mm. (a) same hardware, same sample type, (b) same hardware, different sample type, (c) different hardware, 20X magnification, different sample type. See text for more explanation.

5. Conclusions

In this paper, it was shown that autofocusing of digital holograms of semitransparent biological samples can be achieved by using deep learning. The method was theoretically described and its limitations were investigated.

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References

1. E. Cuche, F. Bevilacqua, and C. Depeursinge, "Digital holography for quantitative phase-contrast imaging," Optics Letters **24**, 291–293 (1999).
2. J. Gillespie and R. A. King, "The use of self-entropy as a focus measure in digital holography," Pattern Recognition Letters **9**, 19–25 (1989).
3. F. Dubois, C. Schockaert, N. Callens, and C. Yourassowsky, "Focus plane detection criteria in digital holography microscopy by amplitude analysis," Optics Express **14**, 5895–5908 (2006).
4. P. Langehanenberg, G. von Bally, and B. Kemper, "Autofocusing in digital holographic microscopy," 3D Research **2**, 1 (2011).
5. I. Bergoënd, T. Colomb, N. Pavillon, Y. Emery, and C. Depeursinge, "Depth-of-field extension and 3D reconstruction in digital holographic microscopy," Proc. SPIE **7390**, 73901C-1 (2009).
6. Y. LeCun, Y. Bengio, and G. Hinton, "Deep learning," Nature **521**, 436–444 (2015).
7. A. Krizhevsky, I. Sutskever, and G. E. Hinton, "Imagenet classification with deep convolutional neural networks," In *Advances in Neural Information Processing Systems*, 1097–1105 (2012).

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