Temporal deep learning classification of digital hologram reconstructions of multicellular samples

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Abstract: Digital holographic microscopy allows label-free capture of the full wavefront of light from an object using a low intensity laser. Using numerical reconstructions as an input to deep convolutional neural networks, detection of tumorigenic samples is feasible.
OCIS codes: 090.1995, 100.6890

1. Introduction

Digital holographic microscopy (DHM) is a label-free, single-shot technique that is well suited for imaging three dimensional objects [1]. A single hologram contains the full wavefront of light from a volume. It can be propagated at any depth of the volume revealing both the in-focus amplitude and phase of any contained transparent objects.

In this paper, we propose to use multiple convolutional neural networks that have been trained by using temporally-different healthy and tumorigenic multicellular samples. After training one can classify an object by using two inputs to the system, namely, a captured digital hologram and the age of the sample. We present the classification capabilities of networks trained with different age samples, and a comparison of results by using amplitude, phase, and a combination of amplitude and phase, as inputs to the networks.

2. Digital holographic microscopy

A magnified digital hologram $H_0(x,y) = |R|^2 + |O|^2 + R^*O + RO^*$ sampled by a digital camera can be propagated at any depth z of the reconstruction volume using the Fresnel approximation [2] as

$$U(x,y;z) = \frac{-i}{\lambda z}\exp(\mathrm{i}kz)\, I_0(x,y) \otimes \exp\left[\mathrm{i}\pi\frac{x^2+y^2}{\lambda z}\right] \tag{1}$$

where λ is the wavelength of the light, $\otimes$ denotes a convolution operation, and $k = 2\pi/\lambda$. The terms R^* and O^* denote the complex conjugates of the reference wave and the object wave, respectively. From the complex-valued reconstruction, the amplitude is defined as

$$A(x,y;z) = \sqrt{\mathrm{Re}[U(x,y;z)]^2 + \mathrm{Im}[U(x,y;z)]^2} \tag{2}$$

3. Deep learning

Deep learning is a computational analysis approach that enables simultaneous analyses at multiple levels of abstraction and can be used efficiently in different applications [3]. Deep convolutional neural networks have been used successfully in various visual object recognition and object detection applications [4–6].

For classification of phase and amplitude reconstructions separately, we used neural networks consisting of five convolutional layers. For networks that take a pair of amplitude and phase reconstructions as input, we used siamese networks [7] consisting of five convolutional layers for each of the amplitude and phase. In siamese networks, the results of the convolutional parts of the network are combined before reaching the fully connected layers.

For training, validation, and testing of our multi-network approach we captured time-lapse data of healthy and tumorigenic multicellular Madin-Darby canine kidney (MDCK) cell clusters by using an off-axis Mach Zehnder digital holographic microscope with 660 nm laser source and 40X microscope objective. This data was partitioned into 6 hour temporal groups that were used to train, validate, and test the approach. An example digital hologram with intensity and phase reconstructions is shown in Fig. 1. Each network and each variant was trained for 50 epochs.

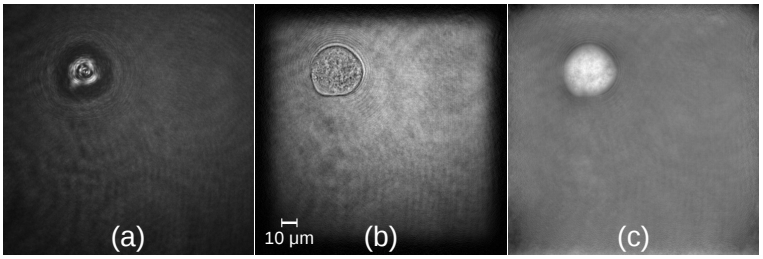

Fig. 1. (a) hologram, (b) intensity, and (c) unwrapped phase reconstructions at depth $z = -11.3$ cm.

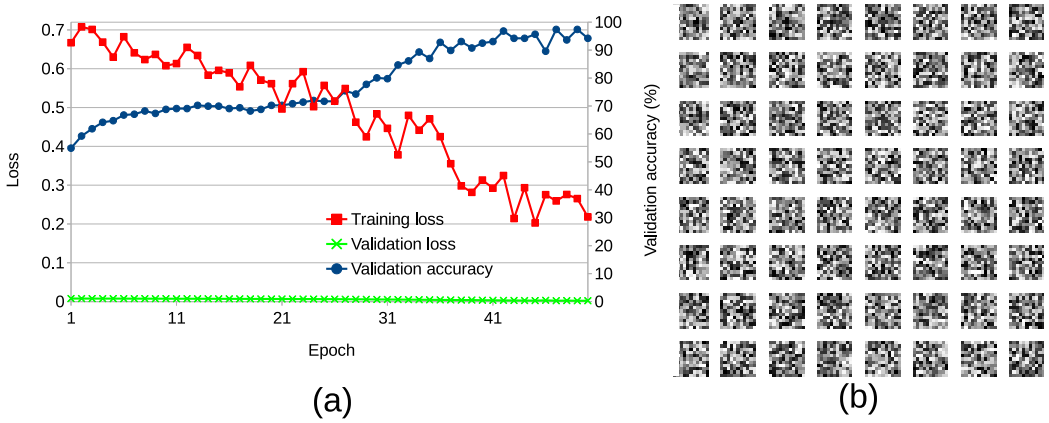

Fig. 2. (a) Training loss, validation loss, and validation accuracy after 50 epochs. (b) Learned weights from the first convolutional layer.

4. Results

Figure 2(a) shows losses together with the validation accuracy for one run using as input amplitude reconstructions from our timelapse sequence during the interval with start time of 108 hours and end time of 114 hours. Fig. 2(b) shows the learned weights from the first convolutional layer. The average testing accuracy of three runs with this particular network and data by using amplitude reconstructions was 92.7%.

Acknowledgements This publication has emanated from research conducted with the financial support of Science Foundation Ireland (SFI) under Grant Number 13/CDA/222, and an Irish Research Council Postgraduate Scholarship.

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