Principal component analysis on time-lapse captured digital holograms of cell clusters

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

[a]*Department of Computer Science, Maynooth University, Maynooth, County Kildare, Ireland*
[b]*Biocenter Oulu, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Finland*
tomi.pitkaaho@cs.nuim.ie, aki.manninen@oulu.fi, tomn@cs.nuim.ie

Abstract: Principal component analysis (PCA) is applied on extracted data of digital holograms of growing cell clusters. We show that PCA can be used to discriminate control and tumorigenic samples.
OCIS codes: 090.1995, 100.6890, 170.6900

1. Introduction

A hologram, H, is an interference pattern of an object wave, O, and a reference wave, R, as $H = |R|^2 + |O|^2 + RO^* + R^*O$ where $|R|^2$ and $|O|^2$ are the object and the reference intensities, respectively [1].

When applying holography to microscopy, digital holographic microscopy (DHM), the object wave can be magnified e.g. using an off-axis configuration with a microscope objective [2] or an in-line setup with a free space propagation [3]. A captured hologram can be focused at different depths of a volume, and the focused numerical reconstruction contains both intensity and quantitative phase information of the imaged object. A digital hologram can be propagated to any depth z using the Fresnel approximation [4]

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

where λ is the wavelength of the light, $\otimes$ denotes a convolution operation and $k = 2\pi/\lambda$. The amplitude and phase components of the complex-valued reconstruction are defined as

$$A(x,y;z) = \{\mathrm{Re}[U(x,y;z)]^2 + \mathrm{Im}[U(x,y;z)]^2\}^{0.5}, \tag{2}$$

$$\phi(x,y;z) = \arctan\{\mathrm{Im}[U(x,y;z)]/\mathrm{Re}[U(x,y;z)]\}, \tag{3}$$

In this paper we show how extracted data from reconstructed phase of time-lapse hologram sequence can be used to discriminate two different cell cluster phenotypes by using principal component analysis (PCA) [5]. We report PCA performance with different age samples that are captured in a time-lapse manner by using an off-axis digital holographic microscope (Fig. 1). The microscope comprises a 660 nm laser source, a 1024×1024 pixel CCD camera with 6.45 μm square pixels, and a 40X microscope objective with 0.7 numerical aperture (Leica HCX PL Fluotar).

We captured both wild-type and tumorigenic Madin-Darby canine kidney (MDCK) cell clusters over six days. A single (x,y) position was imaged approximately every 24 hours. For this paper we used 20 samples from both phenotypes (in total 220 holograms) to demonstrate the discrimination capability of the system.

Cell clusters were reconstructed at in-focus plane that was followed by an automated segmentation. Feature vectors containing eleven quantitative features related to morphology or phase were extracted and normalized to have zero mean and standard deviation of one for each time interval (n=40). Dimension of feature vector was reduced from eleven to two by using PCA [5].

2. Results

None of the eleven extracted features can be used for reliable classification/discrimination on their own. Instead, by combining features with a use of PCA, more accurate discrimination is feasible. Fig. 2 shows first and second principal components of two different datasets, captured at two different imaging intervals. It can be observed that imaging performed at later stage of cell cluster growth leads to more accurate discrimination of two phenotypes.

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.

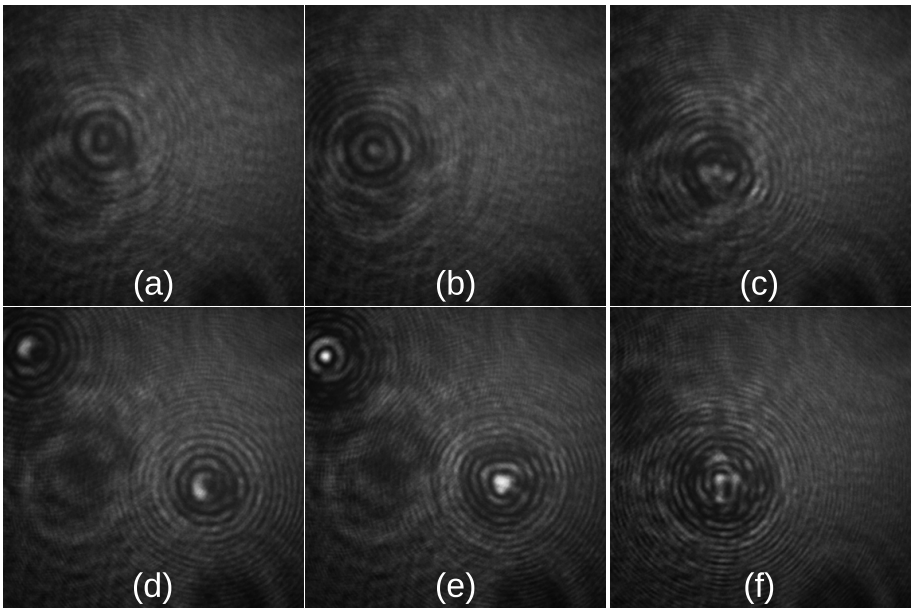

Fig. 1. Example hologram sequence of time-lapse imaging at different imaging times. (a) 0 hours, (b) 24 hours, (c) 48 hours, (d) 72 hours, (e) 96 hours, (f) 120 hours.

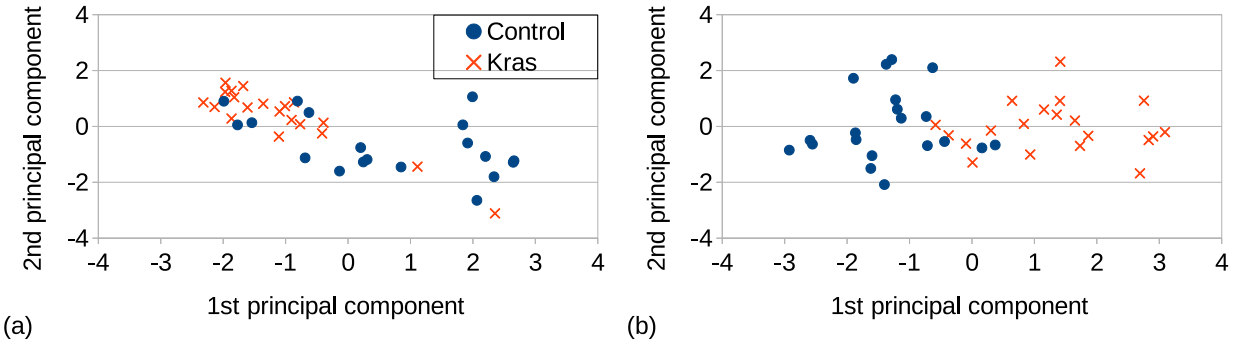

Fig. 2. Principal component analysis at different imaging intervals. Showing 1st and 2nd principal components of extracted data from (a) 24-48 hours and (b) 72-96 hours of the time-lapse imaging.

References

1. D. Gabor, "A new microscopic principle," Nature **161**, 777–778 (1948).
2. E. Cuche, P. Marquet and C. Depeursinge, "Simultaneous amplitude-contrast and quantitative phase-contrast microscopy by numerical reconstruction of Fresnel off-axis holograms," Appl. Opt. **34**, 6994–7001 (1999).
3. H. J. Kreuzer, N. Pomerleau, K. Blagrave, and M. H. Jericho, "Digital in-line holography with numerical reconstruction," in Interferometry 99: Techniques and Technologies, M. Kujawinska and M. Takeda, eds., Proc. SPIE 3744, 65–74 (1999).
4. J. W. Goodman, *Introduction to Fourier Optics,* (Roberts and Company Publishers, 2005).
5. M. E. Tipping and C. M. Bishop. "Probabilistic principal component analysis," Journal of the Royal Statistical Society: Series B (Statistical Methodology) **61.3** 611–622 (1999).