# Monitoring MDCK cell vesicles by digital holographic microscopy and image processing

**Tomi Pitkäaho and Thomas J. Naughton**

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

*tomi.pitkaaho@cs.nuim.ie; tomn@cs.nuim.ie*

**Aki Manninen**

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

*aki.manninen@oulu.fi*

**Abstract:** Digital holographic microscopy and image processing is used to track vesicles of Madin Darby canine kidney cells. Multiple-depth amplitude reconstructions are used as the basis for tracking. Individual vesicle movement in three dimensions is shown.

**OCIS codes:** 090.1995, 100.6890

## 1. Introduction

Vesicle trafficking is a biological process whereby sub-cellular particles transport materials within cells and to/from cells. The healthy behaviour of eukaryotic cells is dependent on the faithful transport of these materials to, and fusion with, the appropriate cellular membrane [1]. Vesicles are critical to a cell's internal organization and are involved in a variety of functions such as metabolism and enzyme storage, and can have fatal consequences when they malfunction. Automated tracking of vesicles is of great interest in cancer research, and cellular biology in general, [2–7]. However, because of their transparent nature, fluorescent tagging is used which can affect functionality, and the high light intensities of 3D microscopes such as confocal microscopes impede their regular use over long timescales. The inability to accurately identify, and track in three-dimensions, thousands of single particles, non-invasively in a label-free manner, via high-throughput microscopy has impeded dynamic studies of vesicle trafficking.

Digital holographic microscopy (DHM) is a label-free, single-shot technique that is well suited for imaging three dimensional objects [8]. DHM has been studied extensively in particle tracking and various different methods for tracking particles have been proposed [9]. We propose to use multiple-depth amplitude reconstructions in the tracking as the amplitude reconstruction of a MDCK cell reveals in-focus vesicle positions accurately and efficiently.

## 2. Digital holographic microscopy

A digital hologram $H_0(x,y) = |R|^2 + |O|^2 + R^*O + RO^*$ that is sampled by a digital camera, can be propagated at any depth $z$ of the reconstruction volume using the Fresnel approximation [10] 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 $\lambda$ 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 intensity component is defined

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

and phase component is defined

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

## 3. Vesicle tracking

MDCK cells can be considered as phase objects that in general do not admit a significant signal in an amplitude reconstruction, however vesicles in the amplitude reconstruction can be distinguished extremely well. Amplitude reconstructions from digital holograms can therefore be used to track vesicles and their position both axially and laterally.

After capturing a time-lapse sequence of living MDCK cells, a hologram from which the tracking will start is chosen. By searching for local minimum along the $z$ axis of the amplitude reconstruction stack one can identify an in-focus vesicle as shown in the Fig. 1. For this, the chosen hologram is reconstructed through a volume using predefined reconstruction depths. The depth map $D(x,y)$ is formed as

$$D(x,y) = \begin{cases} \arg\min_z [V_z(x,y)] \text{if} \min[V_z(x,y)] \geq \tau \\ 0, \text{otherwise} \end{cases} \tag{4}$$

where $V_z(x,y)$ is the amplitude reconstruction volume in which each amplitude reconstruction is multiplied by the mask, and $\tau$ is a manually adjusted threshold. The mask is obtained for each depth by an adaptive thresholding of each amplitude reconstruction. $D(x,y)$ is binarized to $M(x,y)$ by setting all the values greater than 0 to 1, and running a morphological image opening operation on the result. Unique labels are given to each independent region in $M(x,y)$. After extracting each region with a unique label, size of the region is calculated; if the size is below a threshold, $(x',y')$ coordinates of the region are used to obtain a mask $M'(x',y')$ for a vesicle. Median value of $D$ at the same $(x',y')$ coordinates is the starting depth for the tracking.

Tracking can be realized by using a template matching in which the template $T$ is an amplitude reconstruction $M' \cap V_\sigma$. The template with size $w \times h$ is convolved over larger region $C_z$ with size of $w+\eta \times h+\eta$ centered at the same $(x,y)$ than T. The matching is performed on the next frame of the sequence at all the reconstructed depths $z$ as

$$R_z(x,y) = \frac{\sum_{x',y'}[T'(x',y') \cdot C'(x+x',y+y';z)]}{\sqrt{\sum_{x',y'} T'(x',y')^2 \cdot \sum_{x',y'} C'(x+x',y+y';z)^2}} \tag{5}$$

where $T'(x',y') = T(x',y') - 1/(w \cdot h) \cdot \sum_{x'',y''} T(x'',y'')$, $C'(x+x',y+y';z) = C(x+x',y+y';z) - 1/(w \cdot h) \cdot \sum_{x'',y''} C(x+x'',y+y'';z)$, $x' = 0...w-1$, and $y' = 0...h-1$. The best match is extracted by finding the global maximum of $R_z(x,y)$ and the template is updated accordingly for the next tracking round. If the maximum coefficient value is below a threshold, the size of $\eta$ is incremented and therefore the size of $C(x,y;z)$ is increased for the next round.

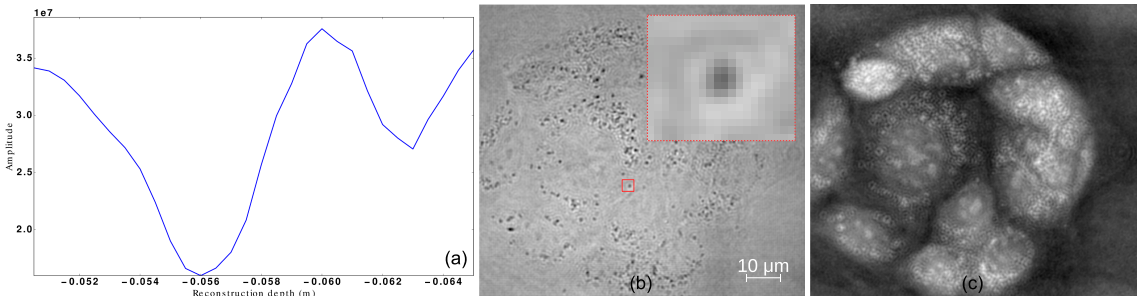


Fig. 1. Focus metric. (a) shows amplitude values of a single pixel of a vesicle at different reconstruction depths; (b) amplitude reconstruction of a MDCK cell hologram at -5.6 cm from the hologram plane where some of the vesicles are in focus, inlet in (b) show an in-focus vesicle; (c) unwrapped phase reconstruction at -5.6 cm from the hologram plane.

## 4. Results

Figure 2 shows tracking result with MDCK cells. MDCK cells were grown in a traditional growth medium and the time-lapse imaging was performed by using an off-axis Mach-Zehnder digital holographic microscope. Imagings were done by using a dry 40X microscope objective with 0.7 numerical aperture (Leica HCX PL Fluotar). One $1024 \times 1024$ (6.45 $\mu$m pixel pitch) hologram was captured every 20 seconds. Aperture apodization was applied on the holograms and the reconstruction volume was reconstructed with 0.5 mm steps from -65.0 mm to -50.5 mm. Adaptive gaussian

thresholding with 40 pixel block size and offset of 40 was applied on the normalised amplitude reconstructions. $\tau$ was set to 50% of the maximum of each amplitude reconstruction, offset of $C$ was 10 pixels, coefficient threshold was 0.95, and $\eta$ was 2 pixels.

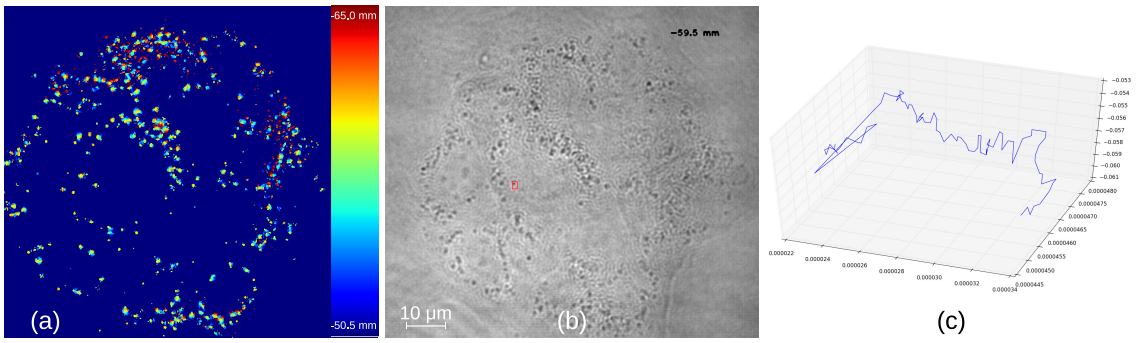


Fig. 2. Result. (a) depth map of the starting frame; (b) last frame of the sequence with red rectangle showing the identified object position; (d) trajectory of the tracked vesicle.

## 5. Conclusions

In this paper, it was shown that vesicle trafficking can be monitored efficiently by using amplitude reconstructions of digital holograms with template matching. The method was theoretically described and experimental results were demonstrated.

**Acknowledgements**

This research was supported by an Irish Research Council Postgraduate Scholarship, Science Foundation Ireland under Grant No. 13/CDA/2224, and Kerttu Saalasti Foundation.

## References

1. K. W. Donovan and A. Bretscher, "Tracking individual secretory vesicles during exocytosis reveals an ordered and regulated process,", JCB **210**, 181–189 (2015).
2. D. M. Yang, C. C. Huang, H. Y. Lin, D. P. Tsai, L. S. Kao, C. W. Chi, and C. C. Lin, "Tracking of secretory vesicles of PC12 cells by total internal reflection fluorescence microscopy," J. Microsc. **209**, 223–227 (2003).
3. C. H. Li, L. Bai, D. D. Li, S. Xia, and T. Xu, "Dynamic tracking and mobility analysis of single GLUT4 storage vesicle in live 3T3-L1 cells," Cell Research **14**, 480–486 (2004).
4. T. C. Ku, Y. N. Huang, C. C. Huang, D. M. Yang, L. S. Kao, T. Y. Chiu, C. F. Hsieh, P. Y. Wu, Y. S. Tsai, and C. C. Lin, "An automated tracking system to measure the dynamic properties of vesicles in living cells," Microsc Res Tech. **70**, 119–34. ( 2007).
5. T. C. Ku, L. S. Kao, C. C. Lin, and Y. S. Tsai, "Morphological filter improve the efficiency of automated tracking of secretory vesicles with various dynamic properties," Microsc. Res. Tech. **72**, 639–649 (2009).
6. Y. Kalaidzidis, "Multiple objects tracking in fluorescence microscopy," J. Math. Biol. **58**, 57–80 (2009).
7. N. Chenouard, et al, "Objective comparison of particle tracking methods," Nat. Methods **11**, 281–289 (2014).
8. E. Cuche, F. Bevilacqua, and C. Depeursinge, "Digital holography for quantitative phase-contrast imaging," Optics Letters **24**, 291–293 (1999).
9. P. Memmolo, L. Miccio, M. Paturzo, G. Di Caprio, G. Coppola, P. A. Netti, and P. Ferraro, "Recent advances in holographic 3D particle tracking," Advances in Optics and Photonics **7**, 713–755 (2015).
10. J. Goodman, *Introduction to Fourier Optics*, Roberts and Company (2005).