In-flow tomographic imaging for single cells analysis

Lisa Miccio[1*], *Daniele* Pirone[1], *Giusy* Giugliano[1], *Michela* Schiavo[1], *Fabrizio* Licitra[1], *Zhe* Wang[1,2], *Francesca* Borrelli[1], *Vittorio* Bianco[1], *Pasquale* Memmolo[1], *Pietro* Ferraro[1]

[1]CNR-ISASI, Institute of Applied Sciences and Intelligent Systems – Via Campi Flegrei 34, Pozzuoli (NA), Italy;
[2] DICMaPI, Department of Chemical, Materials and Production Engineering, University of Naples "Federico II"

Abstract. Gold standard imaging modalities in biological field are based on fluorescence signals providing high specificity and high resolution. Recently, Fluorescence Microscopy has been combined with microfluidics to develop instrumentations called Imaging Flow Cytometers, high-throughput tools that supply bright-field, darkfield and multiple-channels fluorescence images of each single cell passing in the Field Of View (FOV). Nevertheless, Fluorescence Microscopy has some drawbacks as phototoxicity, photobleaching, expensive costs for sample preparations and also the a-priori knowledge of the tags to be used. For these reasons label-free imaging methods greatly increase in the recent years as the Quantitative Phase Imaging (QPI) technologies for microscopy. One of the optical techniques to achieve QPI is Digital Holography. DH in microscopy has several advantages such as the possibility to numerically scan the focal distance, a properties that open to the integration of DH in microfluidics. Indeed DH combined with microfluidic circuits allows to image particles or cells flowing into the FOV at different depths. Here the capabilities of label-free single-cell imaging by DH are presented and their implications on next future biomedical applications discussed. Static or in-flow configurations will be showed describing recent results and perspectives also in combination with Artificial Intelligence architectures for future applications in biomedical and clinical fields.

1 INTRODUCTION

In the realm of biological samples analysis, QPI represents a dynamic tool which provides specimens information to understand the complex biological mechanisms that regulate growth and life of single cells in label-free and non-invasive modality [1]. Indeed, the label-free approach is desirable because it avoids staining procedures that require highly trained personnel. Moreover, label method could alter cell behaviour and cause cell mortality. QPI overcomes these limitations, providing a wealth of information at the single cell level. DH is one of the optical techniques to achieve QPI, adding further advantages such as numerical multiple refocusing capability [2]. Thanks to DH properties it has been demonstrated that living cell, in particular Red Blood Cells behave as microlenses and such property can be exploited to distinguish between healthy and diseased cells [3]. Furthermore, the integration of DH with Flow Cytometry addresses the high-throughput needs of biological applications. The investigation of large volumes in a short time allows collecting comprehensive amount of single cell quantitative information useful for training dataset, permitting the integration of Artificial Intelligence (AI) architectures. Promising results have been demonstrated for classification of cell populations with different characteristics also in relation to diagnostic purposes [4-6]. Recently, the integration with microfluidic platform allows the recording of several views of the same cell exploiting the rotations occurs while it flows along a micro-channel. This approach permits to reconstruct 3D Refractive Index (RI) distribution at single cell level for thousands of cells [7-9]. Here, we show the potential of label-free single cell imaging by DH and future possible applications in biomedical field and clinical practice as for example for anaemia diseases [10].

2 MATERIALS AND METHODS

A typical optical arrangement we implement to record holograms is based on a Mach-Zehnder interferometer in off-axis configuration (Fig. 1). A conventional CMOS camera collects the interference fringe pattern obtained by Beam Splitter (BS2) which recombines the two beams previously split by Beam Splitter (BS1). One of two beams, named object beam, passes through the sample. The scattered contribution is collected by microscope objective (MO1) that magnifies the image. The other beam, named reference beam, is driven through a different path and recombined with the object beam according to the definition of off-axis configuration. When combining the optical setup with a microfluidic platform, we enabled the implementation of DH Tomography Flow Cytometry (DHTFC). Exploiting out-of-focus acquisition capability and the roto-translatory motion of the cells, we operate in

* Corresponding author: lisa.miccio@cnr.it

two possible ways: (i) expansion of real 2D images dataset, (ii) 3D RI distribution reconstruction.

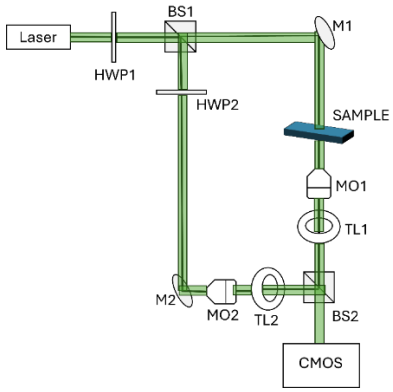

Fig. 1. Schematic view of the Mach-Zehnder interferometer based on off-axis configuration. HWP: half-wave plate; M: mirror; MO: microscope objective; MC: microfluidic channel; TL: tube lens; BS: beam splitter; CMOS: camera.

The Quantitative Phase Maps (QPMs) are retrieved from the acquired holograms by applying several computational steps, such as suppression of zero-order and virtual image, numerical refocusing, aberrations' compensation and phase unwrapping (Fig. 2a). In case of DHTFC a further processing step concerns the estimation of the rolling angles for achieving accurate tomographic reconstructions (Fig. 2b).

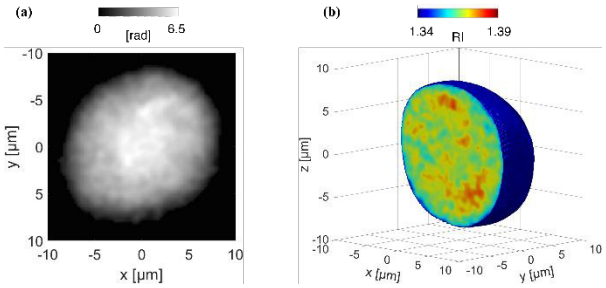

Fig. 2. Tomographic reconstruction of a breast cancer cell (MCF-7). (a) Retrieved QPM (b) Central slice of 3D RI tomographic reconstruction.

3 Results

Potentiality of QPI by means of DH technique has been demonstrated. Mouse Embryonic Fibroblasts in adhesion were investigated for detecting lysosomal compartments [11]. Exploiting the bio-lensing paradigm the possibility to distinguish between different anaemia types was provided [12]. The capabilities of DH for blood component analysis has been achieved also combining 2D holographic imaging and AI [10,13]. Introduction of microfluidics to develop DHTFC paradigm permitted the accurate investigation of subcellular compartments as nuclei, lipid droplets and vacuoles [6,9,14,15]. Furthermore, the innovative approach of real-time exploration of quantitative biophysical parameters for distinct cellular compartments is promising for the development of a 3D metaverse microscope.

4 Conclusions

With our works we demonstrated the advantages of label-free single cell imaging by DH. The flexibility suggests potential applications in future biomedical diagnostic and therapeutic fields. In particular the exploitation of the bio-lensing effect would result effective in the identification of different hereditary anaemias.

This work was supported by a project PRIN 2022 PNRR "Intelligent holographic image-Based differentiaL diagnOsis Of hereDitary anemias" (i-BLOOD); project number P2022WBWXS; CUP B53D23024120001, Finanziato dall'Unione Europea – NextGenerationEU –

References

1. Y. Park, C. Depeursinge, and G. Popescu, Nat. Photonics **12**, 578–589 (2018).
2. P.Y. Liu, L.K. Chin, W. Ser, et al., *Lab Chip* **16(4)**, 634–644 (2016).
3. L. Miccio, P. Memmolo, F. Merola, et al., Nat Commun **6**, 6502 (2015).
4. V.K. Lam, T.C. Nguyen, B.M. Chung, G. Nehmetallah, and C.B. Raub, Cytometry, Part A **93(3)**, 334–345 (2018).
5. E. Moen, D. Bannon, T. Kudo, W. Graf, M. Covert, and D. Van Valen, Nat. Methods **16(12)**, 1233–1246 (2019).
6. M. Ugele, M. Weniger, M. Stanzel, M. Bassler, S.W . Krause, O. Friedrich, O. Hayden, L. Richter, Adv Sci **5,** 1800761 (2018)
7. D. Pirone, J. Lim, F. Merola, et al., Nat. Photon. **16**, 851–859 (2022).
8. Fanous MJ, Ozcan A. *Opto-Electron Adv* **6**, 230083 (2023).
9. Pirone D, Sirico D, Miccio L, Bianco V, Mugnano M et al. *Opto-Electronic Adv* **6**, 220048 (2023)
10. P. Memmolo, G. Aprea, V. Bianco, et al. Biosensors and Bioelectronics **201**, (2022)
11. Giugliano G, Schiavo M, Pirone D, Běhal J, Bianco V, Montefusco S, Memmolo P, Miccio L, Ferraro P, Medina D. L *Cytometry, Part A* **105(5)**, 323–331 (2024).
12. M. Mugnano, P. Memmolo, L. Miccio et al. Analytical Chemistry **90,** 7495-7501 (2018)
13. T. O'Connor, A. Anand, B. Andemariam, and B. Javidi, Biomed. Opt. Express **11**, 4491-4508 (2020)
14. D. Pirone, D. G. Sirico, M. Mugnano, D. Del Giudice, I. Kurelac, B. Cavina, P. Memmolo, L. Miccio, and P. Ferraro, *Biomed. Opt. Express* **13**, 5585-5598 (2022)
15. V. Bianco, M. D'Agostino, D. Pirone, G. Giugliano, N. Mosca, M. Di Summa, G. Scerra, P. Memmolo, L. Miccio, T. Russo, E. Stella, P. Ferraro, *Small Methods* **7**, 2300447 (2023).