



Zhou, Y., Zammit, P., Zickus, V., Carles, G., Taylor, J. and Harvey, A. R. (2017) Video-rate 3D Particle Tracking with Extended Depth-of-field in Thick Biological Samples. In: Computational Optical Sensing and Imaging (COSI 2017), San Francisco, CA, USA, 26-29 Jun 2017, CM3B.3. ISBN 9781557528209 (doi:[10.1364/COSI.2017.CM3B.3](https://doi.org/10.1364/COSI.2017.CM3B.3))

This is the author's final accepted version.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/146753/>

Deposited on: 11 October 2017

Enlighten – Research publications by members of the University of Glasgow
<http://eprints.gla.ac.uk>

Video-rate 3D Particle Tracking with Extended Depth-of-field in Thick Biological Samples

Yongzhuang Zhou, Paul Zammit, Vytautas Zickus, Guillem Carles, Jonathan Taylor
and Andrew R. Harvey*

School of Physics and Astronomy, University of Glasgow, Glasgow, UK G12 8QQ
andy.harvey@glasgow.ac.uk

Abstract: We present a single-aperture 3D particle localisation and tracking technique with a vastly increased depth-of-field without compromising optical resolution and throughput. Flow measurements in a FEP capillary and a zebrafish blood vessel are demonstrated experimentally.

OCIS codes: (180.6900) Three-dimensional microscopy; (100.4999) Pattern recognition, target tracking; (110.1758) Computational imaging; (110.7348) Wave-front encoding; (180.2520) Fluorescence microscopy.

1. Introduction

3D particle localisation and tracking play an important role in research of microfluidics and its applications in life science, lab-on-chip, and many other related research fields. In recent years, several 3D particle localisation techniques have been proposed in literature among which the single-aperture methods based on defocus, astigmatism, multiple focal planes and engineered point-spread functions (PSFs) such as the Double-Helix PSF have been extensively used in micro particle tracking velocimetry (μ -PTV) and localisation-based super-resolution due to their excellent spatial and temporal resolution as well as the simplicity of their implementation [1, 2]. However, current 3D particle localisation and tracking techniques are subject to the seemingly unavoidable trade-off between localisation precision and depth-of-field (DOF). This poses a problem when imaging thick samples such as zebrafish hearts or tracking blood flow in their blood vessels. Considering the importance of zebrafish model for the *in vivo* study of cardiac cell motion and other cardiovascular phenomena[3], we conclude that there is substantial scope in defeating this trade-off.

To this end, in this paper, a novel particle localisation and tracking technique based on wavefront-coding (WC) [4] is proposed. WC is an extended DOF technique originally intended for extended imaging applications. Here, the inherent extension in DOF provided by WC together with a relatively obscure phenomenon associated with the WC PSF (also known as the cubic PSF) have been exploited in order to break the above mentioned trade-off between DOF and localisation precision. Using a 20X, 0.4NA objective with a conventional DOF of 5.8 μm , an axial localisation precision of less than 100 nm is demonstrated over a DOF of 150 μm with a peak signal-to-noise ratio (PSNR) [5] of 38.8 dB. Flow measurements performed in fluorinated ethylene propylene (FEP) capillaries seeded with 0.96 μm fluorescent beads are presented where the results were found to agree well with the theoretical predictions. Lastly, preliminary blood flow mapping measurements performed in live zebrafish are also presented and discussed.

2. Experiments and Result Analysis

The cubic PSF experiences a lateral translation in proportion to the depth of the point emitter which generates it [4, 6]. Measuring this translation, therefore, yields a measurement of the depth of the emitter. This can be accomplished by means of the complementary kernel matching (CKM) configuration discussed in [6, 7]. This consists in acquiring simultaneously two images of the scene with a relative offset in defocus. The relative shift (disparity) between the emitters in each of the two acquired images is then calculated. Finally, this disparity is related to the depth of the emitter through calibration; hence, the 3D position of the emitter is known.

This novel technique was implemented on an inverted microscope (Nikon Eclipse Ti). A glass cubic phase mask (strength parameter $\alpha = 7$) was placed close to the back focal plane of the objective as shown in Fig.1. A lateral beam splitter was used to generate two imaging planes with a distance Δ_i apart as required for CKM. The separation of the two imaging planes was adjustable by the addition of a glass slab with certain thickness. Two PSF encoded images were formed at the same camera sensor (Andor Neo 5.5 sCMOS) and then the captured images were processed to obtain the 3D localisation of each particle.

Fig.2.a shows the PSF of the described system, which was acquired using a single fluorescent bead with an emission wavelength of 520 nm and a diameter of 0.96 μm . When defocused, the image of the point emitter translates along the image diagonal as shown in Fig.2.b, the defocus values are 0, 30 μm , 60 μm respectively. If the acquired images are deconvolved by the correct recovering kernel, the recovered image should look like a point localised at the correct lateral position without translation. Fig.2.c shows recovered images of the particles in Fig.2.b deconvolved with the PSF in Fig.2.a. Because the in-focus image is deconvolved with the in-focus PSF it results in no translation in the recovered image. However, the other two recovered images both yield translations since they are not deconvolved with their corresponding PSFs. Two imaging channels result in different image translations. Only when the correct PSFs are used to perform deconvolution will there be no disparity in two recovered images. Fig.2.d (left) displays a snapshot of a steady laminar flow in a FEP capillary with cubic coded images from both imaging channels while Fig.2.d (right) shows the two recovered images superimposed. Various disparities indicate that the particles are at a range of depths.

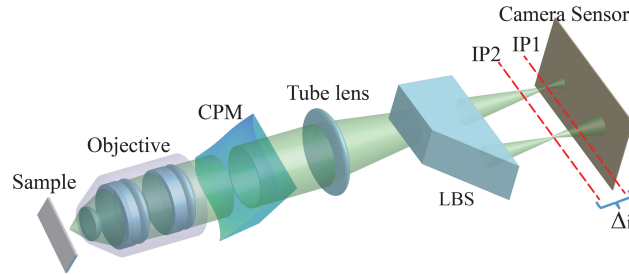


Fig. 1: Schematic of the 3D particle tracking setup using wave-front coding and the CKM method. CPM: cubic phase mask; LBS: lateral beam splitter; IP1: image plane of the 1st imaging channel; IP2: image plane of the 2nd imaging channel. Δ_i is the focal difference between two channels which is about 4.9 waves for our setup.

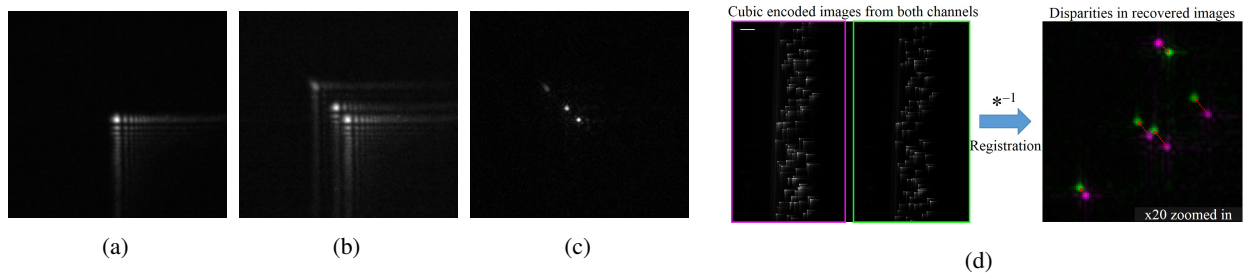


Fig. 2: **a**: image of a single in-focus particle (PSF). **b**: image of the same particle at different depths: $\Delta z=0, 30 \mu\text{m}, 60 \mu\text{m}$ respectively. **c**: images in **b** recovered with the same PSF in **a**. **d**: Left: Cubic encoded images from both imaging channels of a steady laminar flow generated in a FEP capillary. The scale bar is 50 μm . Right: results after deconvolution and two channel registration with magenta spots being recovered image from first imaging kernel and green spots from the second ($\times 20$ zoomed in).

In order to validate the technique, a steady laminar flow in an FEP capillary with an inner diameter of 150 μm was tracked. A syringe pump was used to generate the flow which was seeded with 0.96 μm fluorescent beads. Fig.3.a shows the 3D velocity field in the FEP capillary. As expected, all the fluorescent beads fall inside the capillary inner volume, and the flow velocity varies from the capillary centre to the edge. In fact, since the beads are relatively small and light, we would expect the flow velocity profile to be approximately parabolic: $v(r) = v_m (1 - r^2/R^2)$ with v_m being the maximum velocity and R being the diameter of the capillary. Least-square fitting is applied to the velocity field and the fitted curve profile shows a radial distribution as expected. In addition, the parameter R from curve fitting is 75.6 μm which accurately matches the predicted value of 75 μm . The estimated volume flow rate (by integrating the velocity equation) is also consistent with the reading on the syringe pump.

Zebrafish embryo is an ideal target for blood flow tracking due to its good transparency and low refractive index. *In vivo* particle tracking was performed in a zebrafish blood capillary by injecting 1.04 μm fluorescent beads in its blood stream. Fig.3.b shows an image of a fish after injection with the bright area being the injection point. A thin blood capillary in the tail was observed where the trajectory of a tracer through it is shown in Fig.3.c. From the

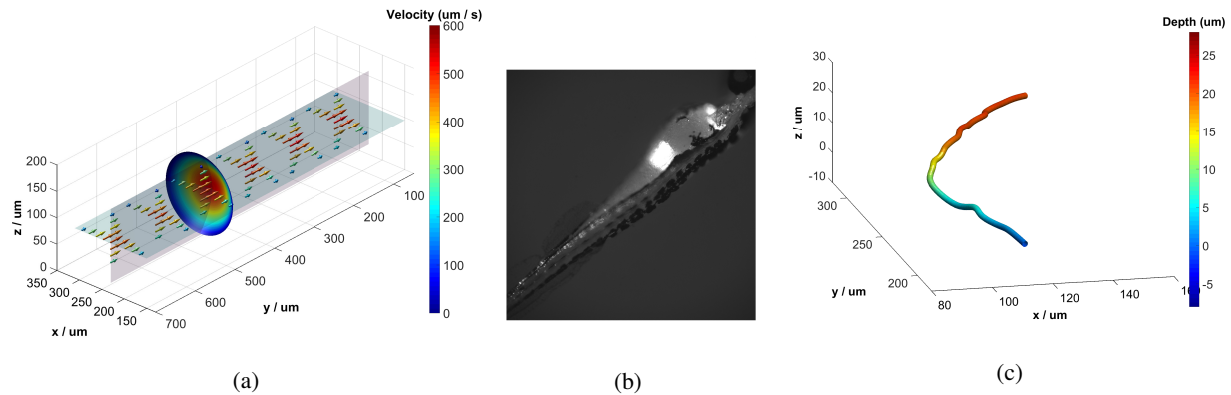


Fig. 3: **a.** 3D velocity field of a steady laminar flow through a FEP capillary with an inner diameter of about $150\ \mu\text{m}$. This result was obtained by averaging 4000 frames (frame rate 10 fps). Vectors on two perpendicular slices are shown, and different colour maps the velocities from 0 to $600\ \mu\text{m}/\text{sec}$. The parabolic curve is a least-square fitting to the velocity vectors at that cross-section. **b.** Zebrafish embryo injected with $1.04\ \mu\text{m}$ fluorescent beads with both bright field and fluorescent illumination. **c.** Tracer trajectory in a live zebrafish blood capillary in its tail with different colour maps the depth.

obtained preliminary results, the spatial extension of the blood capillary and its shape in 3D (including small kinks) are clearly observable. If enough fluorescent beads are tracked (either with a higher seeding concentration or with a longer observing time), a larger vessel can be reconstructed. Moreover, the unprecedented DOF which this technique provides opens up new avenues in the study of high-profile problems such as the blood flow in zebrafish hearts at high frame rate without the need for complex synchronisation equipment [3].

3. Conclusion

We have demonstrated 3D particle localisation and tracking using a novel single-aperture technique which provides an unprecedented increase in DOF. The main advantage of this technique is that the depth range is extended without losing precision. We have obtained a sub-100 nm axial precision over a $150\ \mu\text{m}$ depth range with a PSNR of 38.8 dB. This unique property makes it suitable to image thick biological samples such as mapping blood flow in zebrafish and particle tracking in very thick FEP capillaries as demonstrated experimentally in this paper. Additionally, the deconvolution operation permits higher seeding concentration to be used compared to existing techniques such as astigmatic and defocusing approaches. These advantages are gained using a relatively simple optical setup and post-processing which increase the appeal of the described technique.

References

1. C. Cierpka, and C. J. Khler, "Particle imaging techniques for volumetric three-component (3D3C) velocity measurements in microfluidics," *J. Vis.* **15**, 1–31 (2012).
2. R. Lindken, M. Rossi, and S. Große, and J. Westerweel, "Micro-Particle Image Velocimetry (μ -PIV): Recent developments, applications, and guidelines," *Lab Chip* **9**, 2551–2567 (2009).
3. J. Lu, F. Pereira, S. E. Fraser, M. Gharib, "Three-dimensional real-time imaging of cardiac cell motions in living embryos," *J. Biomed. Opt.* **13**, 014006-014006-8 (2008).
4. E. Dowski, and W. Cathey, "Extended depth-of-field through wave-front coding," *Appl. Opt.* **34**, 1859–1866 (1995).
5. W. K. Pratt, 'Digital image processing, (John Wiley & Sons, 1978).
6. P. Zammit, A. R. Harvey, and G. Carles, "Extended depth-of-field imaging and ranging in a snapshot," *Optica* **1**, 209–216 (2014).
7. Y. Zhou, P. Zammit, and A. R. Harvey, "3D microfluidic particle image velocimetry with extended depth-of-field and a single camera," in *Imaging and Applied Optics 2016*, OSA Technical Digest (online) (Optical Society of America, 2016), paper JT3A.40.