Ultra-fast volumetric imaging of biological processes

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ABSTRACT

Many processes in biology are highly dynamic and take place on the sub-second time scale and occur amid single cells and multi-cellular tissues. Capturing these processes in 3D therefore requires rapid imaging at single cell resolution which is a recurring challenge in microscopy. By combining the concepts of selective-plane illumination and light-field microscopy, we show that previous limitations of both techniques can be overcome and near isotropic single cell resolution can be achieved. To demonstrate our method, we image the beating heart of a Medaka larvae and the blood flow at single cell resolution at up to 200 Hz.

Keywords Light-sheet; light-field; microscopy, biology, fast imaging

1. INTRODUCTION

- Biological processes take place at a variety of different spatial and temporal scales, ranging from subcellular processes at (sub-)microsecond scale to multicellular processes at minute or hour scale. With the development of selective plane illumination microscopy[1] and light-field microscopy[2] (LFM) in the last two decades, developmental processes within embryos as well as brain and muscle activity amid small organisms could be studied at new speeds and durations. These two techniques bridged the gaps between cellular and multicellular as well as spatial and temporal scales. However, up to this day, very fast processes as brain and heart activity remain a great challenge for any fluorescent microscopy technique.
- We combined SPIM and LFM in a single setup and managed to overcome the individual limitations of both techniques in terms of speed and spatial resolution. With our microscope we show that a near-isotropic resolution of ~2 µm in a volume of 300 x 300 x 300 µm³ can be recorded at up to 143 Hz, surpassing the spatio-temporal performances of previous SPIM or LFM implementations significantly.
- With our Dual-view LFM setup we recorded the beating heart of an 8-day post fertilization (dpf) Medaka larvae and were able to resolve the myocardiac tissue layer at single cell resolution. Further, we recorded the blood flow through an adjacent blood vessel and not only resolved single blood cells without motion blur but were also able to track them as throughout the vessel.

2. STATE OF THE ART

Recently rapid volumetric imaging of fast biological processes has been achieved with the help of light-sheet microscopy [3,4] and light-field microscopy [2,5,6]. However, a significant trade-off between resolution and speed is needed with these techniques. While light-sheet microscopy can achieve a comparatively high resolution, its plane by plane recording scheme leads to motion artefacts inside the acquired volume. It is therefore limited to processes that take place over time courses above the image stack acquisition time and typically covers events well that occur on the second to minute scale. Light-field microscopy on the other hand can capture the 3D volume instantaneously and is therefore limited only by the camera speed and the signal present in the fluorescent

sample. The high speed comes at the trade-off of generally low (> µm), as well as varying resolution throughout the acquired volume, with artefacts at the volume's centre and degrading resolution along the detection axis.

Our project tackled the challenge of low resolution and reconstruction artefacts in LFM by introducing a selective volume illumination and dual-view view imaging approach to light-field microscopy. This allowed us to achieve isotropic and cellular resolution while maintaining the ultra-fast volumetric acquisition capability of LFM.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

In this project we combined the strengths of LFM and SPIM in one single setup, profiting from the high speed of LFM and the selective illumination of SPIM. An additional orthogonal light-field detection provided the possibility to fuse the simultaneously recorded volumes into one 3D dataset, thus reducing imaging and reconstruction artefacts through the use of multi-view deconvolution.
The realization of this microscope, named Dual-view LFM, requires a total of 3 objectives, all combined orthogonally to each other (see Figure 1). In this configuration, the two detection objectives were facing downward by 45 degrees in respect to the optical table. The illumination objective was brought in sideways while remaining parallel to the optical table. From this optical arrangement two orthogonal light-fields are recorded simultaneously from a selectively excited volume. By this design, the fluorescence signal of emitters outside the volume of interest is minimized, leading to light-field recordings of higher contrast than in the typically applied widefield illumination scheme. Furthermore, the resulting optical performance of our system yielded an isotropic resolution of 2-3 µm across the imaged volume. The otherwise known varying resolution and strong reconstruction artefacts of LFM[7] around the volume centre were also minimized.

4. PROJECT RESULTS

We have implemented a selective volume illumination dual-view light-field microscope and demonstrated its ability to image highly dynamic processes with cellular isotropic resolution and minimal artefacts. For the first time near isotropic resolution (~ 2 x 3µm) was achieved for instantaneous volumetric imaging (300 x 300 x 300 µm³) of a developing and beating fish larvae's heart at rates of up to 143 Hz [8]. We unambiguously were able to resolve single cells of the myocardiac tissue layer during the active heartbeat, at much better contrast and resolution than standard (epi-) illumination LFM (see Figure 2). Further, single blood cells could be resolved without motion artefacts and tracked throughout the adjacent blood vessels at a rate of up to 200 Hz. This was possible through the combination of a selectively exited volume, paired with orthogonal light-field detection and a sophisticated pipeline, encompassing reconstruction, fusion, and deconvolution algorithms. The pipeline was optimized for cluster computation and required only minimal human interaction. The simultaneous use of two objectives in Dual-view LFM effectively doubles the light collection efficiency and eliminates spatio-temporal ambiguity associated with 3D imaging approaches based on sequential recording of image planes. Currently the upper limit in imaging speed is given by the camera read-out time (4ms) and is not fluorescence limited (exposure time 1ms). Thus, we foresee that future camera developments featuring multi-area read-out architectures can further increase the overall imaging speed towards the volumetric kHz regime. This would make Dual-view LFM an attractive tool to record voltage dynamics on millisecond time scales in 3D within small neuronal networks.

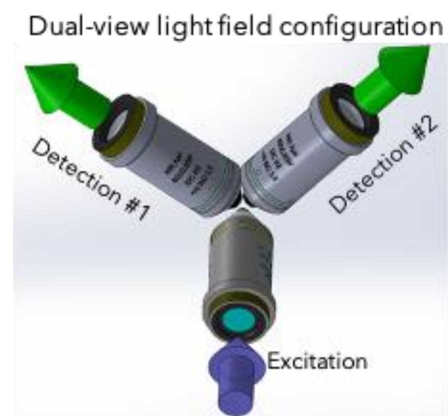

Fig. 1. Principle of dual-view LFM. Objective configuration showing excitation in blue and fluorescence detection in green.

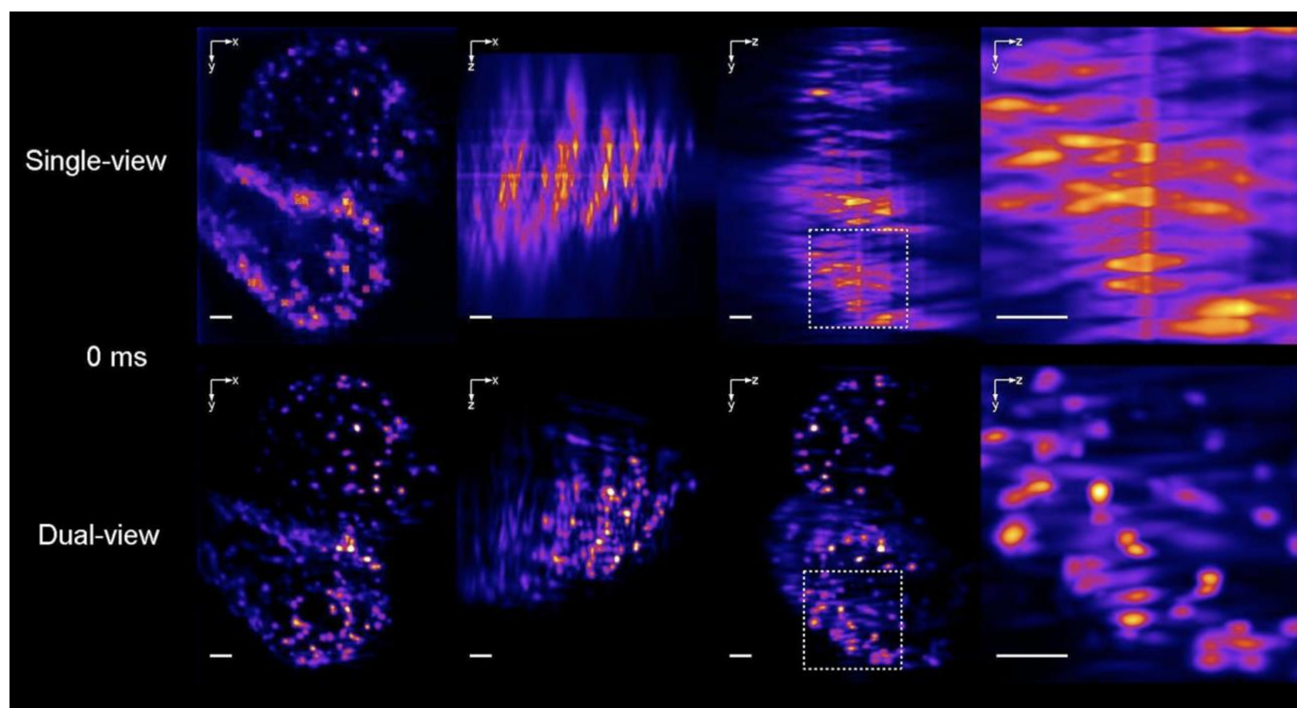

Fig. 2. Ultrafast volumetric imaging of heart beating in the juvenile Medaka fish using Dual-view LFM. Top: Maximum intensity projections of single-view light-field images of the heart (*myl7::H2B-eGFP*), at 143Hz volume rate over 300x300x300µm. Low resolution and prominent artefacts are apparent. Bottom: With dual-view Iso-LFM, single-cell resolution is clearly restored, and the artefact plane largely removed (c.f. zoom-ins denoted by white dashed boxes).

5. FUTURE PROJECT VISION

The original project plan was to utilize SPAD array sensors for this project. However, due to the unavailability of initially proposed sensor, we utilized commercial sCMOS cameras for our work as described above. As the main project lead (Lars Hufnagel) has left EMBL and academia, we do not foresee to continue the original project during an ATTRACT Phase 2.

We are currently working on artificial intelligence assisted data and image processing ideas that are connected to the above described work[9]. If deemed interesting we will consider applying with a new project and new project leads for Phase 2.

6. ACKNOWLEDGEMENT

We would like to thank the EMBL Heidelberg mechanical and electronic workshop and the IT-department for support, as well as C. Tischer (EMBL ALMF) for help. J.G. was supported by a Heidelberg Research Center for Molecular Medicine (HRCMM) Career Development Fellowship (CDF) and by the Deutsche Herzstiftung e.V. (S/02/17). N.N acknowledges support from VINNOVA and Åke Wiberg foundation. This work was supported by the European Molecular Biology Laboratory and the project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222.

7. REFERENCES

1. Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J. & Stelzer, E. H. K. Optical Sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy. *Science (80-.).* **305**, 1007 LP – 1009 (2004).
2. Prevedel, R. *et al.* Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy. *Nat. Methods* **11**, 727–30 (2014).
3. Fahrbach, F. O., Voigt, F. F., Schmid, B., Helmchen, F. & Huisken, J. Rapid 3D light-sheet microscopy with a tunable lens. *Opt. Express* **21**, 21010 (2013).
4. Mickoleit, M. *et al.* High-resolution reconstruction of the beating zebrafish heart. *Nat. Methods* **11**, 919–922 (2014).
5. Pégard, N. C. *et al.* Compressive light-field microscopy for 3D neural activity recording. *Optica* **3**, 517 (2016).
6. Nöbauer, T. *et al.* Video rate volumetric Ca2+ imaging across cortex using seeded iterative

demixing (SID) microscopy. *Nat. Methods* **14**, 811–818 (2017).
7. Broxton, M. *et al.* Wave optics theory and 3-D deconvolution for the light field microscope. *Opt. Express* **21**, 25418 (2013).
8. Wagner, N. *et al.* Instantaneous isotropic volumetric imaging of fast biological processes. *Nat. Methods* **16**, 497–500 (2019).
9. Wagner, N. *et al.* Deep learning-enhanced light-field imaging with continuous validation. *bioRxiv* 2020.07.30.228924 (2020). doi:10.1101/2020.07.30.228924