A new angle on light-sheet microscopy and real-time image processing

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1 Introduction

general intro to light-sheet challenges in mammalian imaging incubation light sensitivity microdrop culture challenges in data handling extremely high data rate preprocessing necessary image fusion image compression

2 DualMouse SPIM

3 Real time image processing

GPU-based image fusion

B^{3}D image compression algorithm

other papers cited here huisken [1] muvi spim [2]

4 New scientific results

Thesis I. *I have designed and constructed a new light-sheet microscope suitable for high resolution near isotropic imaging of delicate samples. A novel arrangement of two high numerical aperture objectives in 120 degrees allows for near isotropic resolution while increasing light collection efficiency by a factor of two.*

Corresponding publications: [J1],[J2], [J3]

Live imaging of light sensitive specimens, such as a developing mouse embryo is a challenging task

Thesis II. *I have developed a GPU-based image processing pipeline for multi-view light-sheet microscopy that enables real time fusion of opposing views.*

Corresponding publications: [C1], [C2], [C3]

Thesis III. *I have developed a new image compression algorithm that enables noise dependent lossy compression of light microscopy images, and can reach a compression ratio of 100 fold while preserving the results of downstream data analysis steps. A fast CUDA implementation allows for real-time image compression of high-speed microscopy images.*

Corresponding publications: [J4], [C1], [C2], [C3]

Since many high-speed microscopy methods generate immense amounts of data, easily reaching terabytes per experiment, image compression is especially important to efficiently deal with such datasets. Existing compression methods suitable for microscopy images are not able to deal with the high data rate of modern sCMOS cameras ($\sim 800\,\mathrm{MB/s}$).

I developed $\mathrm{B}^3\mathrm{D}$, a GPU-based parallel image compression algorithm capable of over $1\,\mathrm{GB/s}$ throughput, allowing live image compression. To further reduce the data size, I developed a noise dependent lossy compression that only modifies the data in a deterministic manner. The allowed differences for each pixel can be specified as a proportion of the inherent image noise, accounting for photon shot noise and camera readout noise. Due to the use of pixel prediction, the subjective image quality is higher than for other methods that simply quantize the square root of the images.

Thesis IV. *I have shown that within noise level compression does not affect the results of most commonly used image processing tasks, and it allows a factor of 4 increase in compression ratio compared to lossless methods.*

Corresponding publications: [J4], [C1], [C2], [C3]

As data integrity in microscopy is paramount for

5 Application of the results

Both the new DualMouse-SPIM microscope and the GPU-based image processing and compression pipeline have direct applications in light-sheet imaging of embryonic development.

Multiple potential collaborators indicated their interest in using the DualMouse-SPIM for their studies in mouse embryonic development. The Hiiragi group, focusing on symmetry breaking events in the pre-implantation and early post-implantation stages would like to use this system for imaging larger specimens from multiple direction, which is not possible on their current microscopes, and could allow them to observe previously unknown mechanisms. The Ellenberg group is interested in investigating chromosome missesgregation mechanisms in the first few divisions during embryonic development. The increased axial resolution of this system will allow to track each individual chromosome during the division process, which was not possible on their current setup due to the insufficient axial resolution.

The GPU-based image processing pipleline, especially the 2D fusion of opposing views is already being used on our lab's workhorse microscope, the MuVi-SPIM. Being able to fuse the two views of the opposing

objectives during imaging not only results in considerable storage space savings, but significantly speeds up the data analysis as well.

The image compression algorithm, B^3D, although was developed with light-sheet microscopy in mind, has a more wide-spread use-case. Any kind of high-speed, high-throughput light-microscopy experiment can benefit form the massive data reduction offered by the within noise level mode. Since the compression can also be done immediately during imaging, not only the storage requirements, but the data bandwidth is reduced as well, which renders the use of high performance RAID arrays and 10 Gbit networks unnecessary, further reducing costs. Due to the similarly high decompression speed, reading the data is also accelerated, which can be beneficial for data browsing and 3D rendering applications. Several companies of different fields already expressed their interest in the compression library, such as Bitplane AG (3D data analysis and visualisation), Luxendo GmbH (light-sheet microscopy), and Hamamatsu Photonics K.K (camera and sensor manufacturing).

The author's publications

[J1] Gustavo de Medeiros, Bálint Balázs, and Lars Hufnagel. "Light-sheet imaging of mammalian development". *Seminars in Cell & Developmental Biology*. Mammalian development 55 (July 2016), pp. 148–155. DOI: 10.1016/j.semcdb.2015.11.001 (cit. on p. 1).

[J2] Petr Strnad, Stefan Gunther, Judith Reichmann, Uros Krzic, Balint Balazs, Gustavo de Medeiros, Nils Norlin, Takashi Hiiragi, Lars Hufnagel, and Jan Ellenberg. "Inverted light-sheet micro-

scope for imaging mouse pre-implantation development". *Nature Methods* 13.2 (Feb. 2016), pp. 139–142. DOI: 10.1038/nmeth.3690 (cit. on p. 1).

[J3] Patrick Hoyer, Gustavo de Medeiros, Bálint Balázs, Nils Norlin, Christina Besir, Janina Hanne, Hans-Georg Kräusslich, Johann Engelhardt, Steffen J. Sahl, Stefan W. Hell, and Lars Hufnagel. "Breaking the diffraction limit of light-sheet fluorescence microscopy by RESOLFT". *Proceedings of the National Academy of Sciences* 113.13 (Mar. 2016), pp. 3442–3446. DOI: 10.1073/pnas.1522292113 (cit. on p. 1).

[J4] Balint Balazs, Joran Deschamps, Marvin Albert, Jonas Ries, and Lars Hufnagel. "A real-time compression library for microscopy images". *bioRxiv* (July 2017), p. 164624. DOI: 10.1101/164624 (cit. on pp. 2, 3).

The author's others publications

[J5] Zoltán Jakus, Edina Simon, Bálint Balázs, and Attila Mócsai. "Genetic deficiency of Syk protects mice from autoantibody-induced arthritis". *Arthritis and Rheumatism* 62.7 (July 2010), pp. 1899–1910. DOI: 10.1002/art.27438.

[J6] Balázs Győrffy, Zsombor Benke, András Lánczky, Bálint Balázs, Zoltán Szállási, József Timár, and Reinhold Schäfer. "RecurrenceOnline: an online analysis tool to determine breast cancer recurrence and hormone receptor status using microarray data". *Breast Cancer Research and Treatment* (July 2011). DOI: 10.1007/s10549-011-1676-y.

[J7] Weiwei Shi, Balint Balazs, Balazs Györffy, Tingting Jiang, W. Fraser Symmans, Christos Hatzis, and Lajos Pusztai. "Combined analysis of gene expression, DNA copy number, and mutation profiling data to display biological process anomalies in individual breast cancers". *Breast Cancer Research and Treatment* 144.3 (Mar. 2014), pp. 561–568. DOI: 10.1007/s10549-014-2904-z.

The author's conference presentations

[C1] Bálint Balázs, Marvin Albert, and Lars Hufnagel. "GPU-based image processing for multiview microscopy data". *Light Sheet Fluorescence Microscopy International Conference*. Sheffield, UK, Sept. 2016 (cit. on pp. 2, 3).

[C2] Bálint Balázs, Marvin Albert, and Lars Hufnagel. "GPU-based image processing for multi-view microscopy data". *Focus on Microscopy*. Taipei, Taiwan, Mar. 2016 (cit. on pp. 2, 3).

[C3] Bálint Balázs, Marvin Albert, and Lars Hufnagel. "GPU-based image processing for multi-view microscopy data". *Focus on Microscopy*. Bordeaux, France, Apr. 2017 (cit. on pp. 2, 3).

References cited in the thesis

[1] Jan Huisken, Jim Swoger, Filippo Del Bene, Joachim Wittbrodt, and Ernst H. K Stelzer. "Optical Sectioning Deep Inside Live

Embryos by Selective Plane Illumination Microscopy". *Science* 305.5686 (2004), pp. 1007–1009 (cit. on p. 1).

[2] Uros Krzic, Stefan Gunther, Timothy E. Saunders, Sebastian J. Streichan, and Lars Hufnagel. "Multiview light-sheet microscope for rapid in toto imaging". *Nature Methods* 9.7 (July 2012). MuVi-SPIM, pp. 730–733. DOI: 10.1038/nmeth.2064 (cit. on p. 1).