

Light-sheet mikroszkópia és valós idejű képfeldolgozás új szemszögből

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A Ph.D. disszertáció tézisei

Budapest, 2017

1 Introduction

Imaging techniques such as microscopy are one of the most extensively used tools in medical and biological research. The reason for this is simple: visualizing something invisible to the naked eye is an extremely powerful way to gain insight to its inner workings. Our brain has evolved to receive and process a multitude of signals from various sensors, and arguably the most powerful of these is vision.

As a branch of optics, microscopy (from ancient Greek mikros, “small” and skopein, “to see”) is based on observing the interactions of light with an object of interest, such as a cell. To be able to see these interactions, the optics of the microscope magnifies the image of the sample, which can be recorded on a suitable device. For the first microscopes in the 17th century, this was just an eye at the end of the ocular, and the recording is a drawing of the observed image [1].

Microscopy is a truly multidisciplinary field: even in its simplest form, just using a single lens, the principles of physics are applied to gain a deeper understanding of biology and nature. Today, microscopy encompasses most of natural sciences and builds on various technological advancements. While physics and biology are still in the main focus, chemistry (fluorescent molecules), engineering (automation) and computer science (image analysis) are all integrated in a modern microscopy environment.

2 Challenges in three dimensional imaging of live specimens

Challenges in image acquisition

Live imaging is indispensable to understand the processes during embryonic development. In an ideal setting, the ultimate microscope would be able to record a continuous, 3D, multicolor dataset of any biological process of interest with the highest possible resolution. Unfortunately this is not possible; due to several limitations in physics and biology, a compromise is necessary. The diffractive nature of light, the lifetime of fluorescent probes and the photosensitivity of biological specimens all require microscopy to be able to adapt to answer the question at hand. In order to acquire useful data one has to choose a tradeoff between spatial and temporal resolution, and signal contrast, while making sure the biology is not affected by the imaging process itself (Figure 1a) [2].

Light-sheet fluorescence microscopy (LSFM), also called single plane illumination microscopy (SPIM) [3], is a relatively new addition to the arsenal of tools that comprise light microscopy methods, and is especially suitable for live imaging of embryonic samples over extended periods of time [4–7]. It is also easily adapted to the sample, allowing to image a large variety of specimens, from entire organs [8], to the subcellular processes occurring inside cultured cells [9].

Even though light-sheet microscopy offers intrinsic optical sectioning To achieve isotropic 3D resolution, multiple views from different directions can be combined [10]. This is most commonly achieved by embedding the sample in an aqueous gel which offers unrestricted view from multiple directions, and a stiff environment to position the sample

[11]. Gel embedding, however is not always possible. Delicate samples, such as mouse embryos, require a very specific environment for proper development [12], which makes sample rotation impossible.

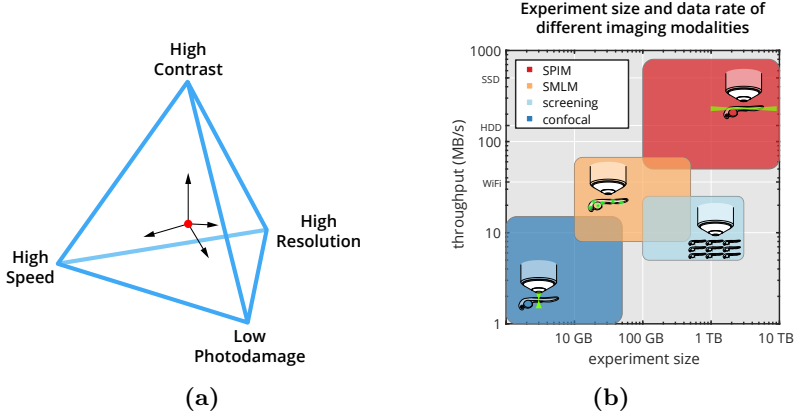


Figure 1: Challenges in microscopy. (a) Tradeoffs in fluorescence microscopy for live imaging. When optimizing the imaging conditions (red dot), a tradeoff has to be made between resolution, contrast, and imaging speed, while avoiding photodamage. Adapted from [2]. (b) Experiment sizes and data rate of different imaging modalities. Comparison of single-plane illumination microscopy (SPIM, red), high-content screening (light blue), single molecule localization microscopy (SMLM, orange) and confocal microscopy (blue) by typical experiment size and data production rate.

Challenges in image processing

When using any kind of microscopy in research, image processing is a crucial part of the workflow. This is especially true for light-sheet microscopy, since it is capable of imaging the same specimen for multiple days, producing immense amounts of data. A single overnight experiment of *Drosophila* development (which is a very typical use-case for light-sheet microscopy) can produce multiple terabytes of data.

Apart from light-sheet microscopy, many other microscopy modalities are also suffering from this problem. Methods, such as high content screening [13–15], where tens of thousands of different genotypes are imaged generating millions of images; and single molecule localization microscopy (SMLM) [16–18], where just a single plane of a sample is imaged hundreds of thousands of times to acquire super-resolved images.

Not only these methods are capable of generating data extremely fast, but with the sustained high data rate a single experiment can easily reach multiples of terabytes (Figure 1b). Handling this amount of data can quickly become the bottleneck for many discoveries, which is a more and more common issue in biological research [19–21].

3 Új tudományos eredmények

This work tackles the previously outlined challenges in light-sheet microscopy: high resolution live imaging of delicate samples, such as mouse embryos, and real-time image processing and compression of large light-sheet datasets.

Tézis I. *Megterveztem es megépítettem egy light-sheet mikroszkópot, ami érzékeny minták nagy felbontású vizsgálatára alkalmas. A két darab, magas numerikus apertúrájú objektív 120 fokban történő elhelyezése, egy megdöntött light-sheettel kombinálva, izotróp 3D felbontású képalkotásra ad lehetőséget, miközben a fény begyűjtésének hatékonyságát kétszeresre növeli meg.*

Kapcsolódó publikációk: [J1],[J2], [J3]

A Dual Mouse-SPIM egy újszerű megközelítést képvisel a light-sheet mikroszkópiában. A 120 fokban elhelyezett magas numerikus apertúrájú objektívek nemcsak a felbontás javítását eredményezi a hagyományos 90 fokos elrendezéssel szemben, hanem a nagyobb detektálási szögnek köszönhetően a fény begyűjtése kétszer hatékonyabban történik. Ez legfőképpen fényérzékeny minták esetén, mint például egér embriók esetén lehet előnyös, mivel a fototoxikus hatások lecsökkenthetők, míg a kontraszt megmarad. Mindét objektív használható megvilágításra és detektálásra is, ami lehetővé teszi a forgatás nélküli többnézetes felvételt.

A mikroszkóp részeként megterveztem egy egyedi nyalábosztó egységet, ami lehetővé teszi csupán egyetlen galvo-szkennert használatát a light-sheet generáláshoz mindkét objektív esetén. Továbbá egy olyan testreszabott detektálás-összeolvasztó egységet is megterveztem, ami lehetővé teszi egyetlen kamera használatát mindkét nézetből.

Karakterizáltam a mikroszkóp optikai tulajdonságait, meghatározva a megvilágítási profilt és a pontátviteli függvényt (PSF). A $95\text{ }\mu\text{m}$ -es látómező egy $3.6\text{ }\mu\text{m}$ vastagságú light-sheettel van egyenletesen megvilágítva. A mintaként használt gömböcskék két irányból történő képalkotása 314 nm laterális és 496 nm axiális felbontást eredményezett. Ez egy 2.67-szeres javulás egyetlen lencse axiális felbontásához képest. Ezen kívül demonstráltam a mikroszkóp képességét *Drosophila* embriókról és az egér zigótákról készült felvételekkel.

Tézis II. *Kidolgoztam egy GPU-alapú képfeldolgozási pipeline-t többnézetes light-sheet mikroszkópiára, amely lehetővé teszi a szemközti nézetek valós időbeni összeolvasztását.*

Kapcsolódó publikációk: [C1], [C2], [C3]

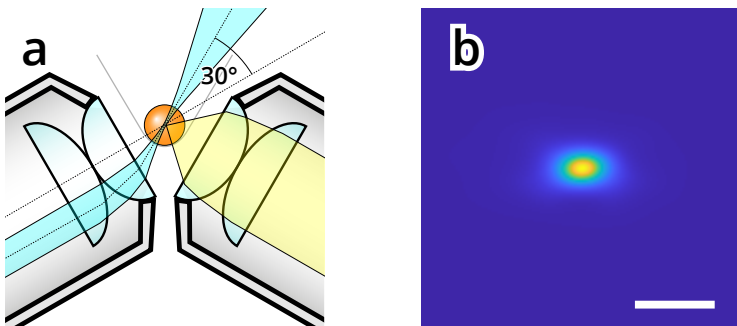


Figure 2: Dual Mouse-SPIM concept. (a) Objective configuration. Both objectives can be used sequentially for both illumination and detection. (b) Combined PSF of the two views. Average of 12 fluorescent beads. Scale bar, 1 μm .

Kidolgoztam egy GPU-alapú képfeldolgozási pipeline-t, mely közvetlenül integrálható a LabVIEW alapú univerzális mikroszkóp vezérlő rendszerünkbe. A pipeline jelenleg lehetővé teszi a háttér levonását és a háttér maszkolását, továbbá képes az azonos sík szemközti nézeteinek azonnali összeolvasztására. Megmutattam, hogy lehetséges a szemközti nézetek regisztrálásának 3D problémáját 2D problémára redukálni, anélkül, hogy ez negatív hatással lenne a képminőségre és a felbontásra. Ez jelentősen lecsökkenti a szükséges számítási kapacitást és lehetővé teszi a CUDA textúrák alkalmazását és a valós időbeni összeolvasztást. Ennek a megoldásnak a feldolgozási sebessége 138 fps, ami 18.3-szoros növekedés az egyszálú CPU verzióhoz képest.

Tézis III. *Kifejlesztettem egy képtömörítési algoritmust, ami lehetővé teszi a fénymikroszkópokkal nyert képek zaj-függő veszteséges tömörítését és akár százszoros tömörítési arányt is elérhet, megőrizve a későbbi adatfeldolgozó lépések eredményeit. A gyors CUDA imple-*

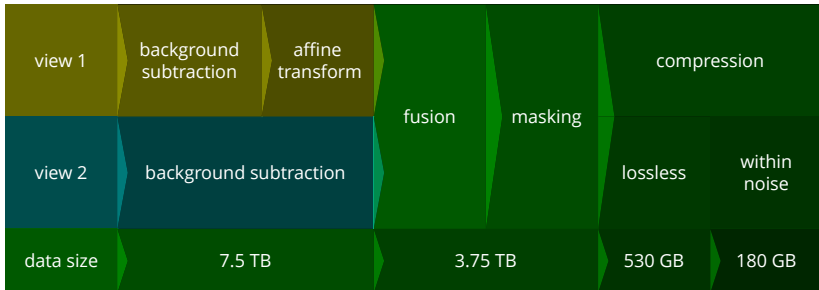


Figure 3: Real-time image processing pipeline for multi-view light-sheet microscopy.

mentáció lehetővé teszi a nagysebességű kamerák képeinek valós idejű tömörítését.

Kapcsolódó publikációk: [J4], [C1], [C2], [C3]

Mivel számos nagysebességű mikroszkópia eljárás hatalmas mennyiségű adatot generál, kísérletenként akár terabájtokat elérve, a képtömörítés kiemelt szerepet kap ilyen adathalmazok esetén. A jelenleg használt, mikroszkópos képekkel kompatibilis képtömörítési eljárások nem képesek a modern sCMOS kamerák nagy adatsebességével megküzdeni (~ 800 MB/s).

I developed a GPU-based parallel image compression algorithm called B³D, capable of over 1 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.

Tézis IV. *I have shown that within noise level compression does not significantly affect the results of most commonly used image processing tasks, and it allows a $3.32\times$ average increase in compression ratio compared to lossless mode.*

Kapcsolódó publikációk: [J4], [C1], [C2], [C3]

As data integrity in microscopy is paramount for drawing the right conclusions from the experiments, using a lossy compression algorithm might be controversial. I have shown that the within noise level (WNL) mode of B³D does not significantly affect the results of several commonly used image analysis tasks. For light-sheet microscopy data I have shown that WNL compression introduces less variation to the image than the photon shot noise. When segmenting nuclei of *Drosophila* embryos and membranes of *Phallusia* embryos, the overlap of the segmented regions of uncompressed and WNL compressed datasets were 99.6% and 94.5% respectively, while compression ratios were 19.83 for *Drosophila* and 40.01 for *Phallusia* embryos. For single molecule localization microscopy data I have shown that WNL compression only introduces 4% increase in localization uncertainty, while the average compression ratio is increased from 1.44 (lossless) to 4.96 (WNL). I have also shown that change in localization error due to the compression does not depend on the SNR of the input images.

4 Application of the results

Both the new Dual Mouse-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 Dual Mouse-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 missegregation 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 pipeline, 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, B³D, 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 from 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 ap-

plications. Several companies of different fields already expressed their interest in the compression library, including 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.semcd.2015.11.001 (cit. on p. 4).
- [J2] Petr Strnad, Stefan Gunther, Judith Reichmann, Uros Krzic, Balint Balazs, Gustavo de Medeiros, Nils Norlin, Takashi Hiragi, Lars Hufnagel, and Jan Ellenberg. “Inverted light-sheet microscope for imaging mouse pre-implantation development”. *Nature Methods* 13.2 (Feb. 2016), pp. 139–142. DOI: 10.1038/nmeth.3690 (cit. on p. 4).
- [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. 4).

- [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. 7, 8).

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 Györffy, Zsombor Benke, András Lánckzy, 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. 5, 7, 8).
- [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. 5, 7, 8).
- [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. 5, 7, 8).

References cited in the thesis

- [1] Robert Hooke. *Micrographia: or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses. With Observations and Inquiries Thereupon*. London: J. Martyn and J. Allestry, 1665 (cit. on p. 1).
- [2] P. Philippe Laissue, Rana A. Alghamdi, Pavel Tomancak, Emmanuel G. Reynaud, and Hari Shroff. “Assessing phototoxicity in live fluorescence imaging”. *Nature Methods* 14.7 (July 2017), pp. 657–661. DOI: 10.1038/nmeth.4344 (cit. on pp. 2, 3).
- [3] Jan Huiskens, 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. 2).

- [4] Philipp J Keller and Ernst HK Stelzer. “Quantitative in vivo imaging of entire embryos with Digital Scanned Laser Light Sheet Fluorescence Microscopy”. *Current Opinion in Neurobiology* 18.6 (Dec. 2008). review, pp. 624–632. DOI: 10.1016/j.conb.2009.03.008 (cit. on p. 2).
- [5] Jan Huiskens and Didier Y. R. Stainier. “Selective plane illumination microscopy techniques in developmental biology”. *Development* 136.12 (June 2009), pp. 1963–1975. DOI: 10.1242/dev.022426 (cit. on p. 2).
- [6] Michael Weber and Jan Huiskens. “Light sheet microscopy for real-time developmental biology”. *Current Opinion in Genetics & Development* 21.5 (2011), pp. 566–572. DOI: 10.1016/j.gde.2011.09.009 (cit. on p. 2).
- [7] Raju Tomer, Khaled Khairy, and Philipp J Keller. “Shedding light on the system: studying embryonic development with light sheet microscopy”. *Current Opinion in Genetics & Development. Developmental mechanisms, patterning and evolution* 21.5 (Oct. 2011). Review from 2011, pp. 558–565. DOI: 10.1016/j.gde.2011.07.003 (cit. on p. 2).
- [8] Hans-Ulrich Dodt, Ulrich Leischner, Anja Schierloh, Nina Jährling, Christoph Peter Mauch, Katrin Deininger, Jan Michael Deussing, Matthias Eder, Walter Zieglgänsberger, and Klaus Becker. “Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain”. *Nature Methods* 4.4 (Mar. 2007), pp. 331–336. DOI: 10.1038/nmeth1036 (cit. on p. 2).

- [9] Bi-Chang Chen et al. “Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution”. *Science* 346.6208 (Oct. 2014), p. 1257998. DOI: 10.1126/science.1257998 (cit. on p. 2).
- [10] Stephan Preibisch, Fernando Amat, Evangelia Stamatakis, Mikhail Sarov, Robert H. Singer, Eugene Myers, and Pavel Tomancak. “Efficient Bayesian-based multiview deconvolution”. *Nature Methods* 11.6 (June 2014), pp. 645–648. DOI: 10.1038/nmeth.2929 (cit. on p. 2).
- [11] 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. 3).
- [12] Adam S. Doherty and Richard M. Schultz. “Culture of Preimplantation Mouse Embryos”. *Developmental Biology Protocols. Methods in Molecular Biology™*. DOI: 10.1385/1-59259-685-1:47. Humana Press, 2000, pp. 47–52 (cit. on p. 3).
- [13] Anne E. Carpenter and David M. Sabatini. “Systematic genome-wide screens of gene function”. *Nature Reviews Genetics* 5.1 (Jan. 2004), pp. 11–22. DOI: 10.1038/nrg1248 (cit. on p. 4).
- [14] Christophe J. Echeverri and Norbert Perrimon. “High-throughput RNAi screening in cultured cells: a user’s guide”. *Nature Reviews Genetics* 7.5 (May 2006), pp. 373–384. DOI: 10.1038/nrg1836 (cit. on p. 4).

- [15] Rainer Pepperkok and Jan Ellenberg. “High-throughput fluorescence microscopy for systems biology”. *Nature Reviews Molecular Cell Biology* 7.9 (Sept. 2006), pp. 690–696. DOI: 10.1038/nrm1979 (cit. on p. 4).
- [16] Eric Betzig, George H. Patterson, Rachid Sougrat, O. Wolf Lindwasser, Scott Olenych, Juan S. Bonifacino, Michael W. Davidson, Jennifer Lippincott-Schwartz, and Harald F. Hess. “Imaging Intracellular Fluorescent Proteins at Nanometer Resolution”. *Science* 313.5793 (Sept. 2006), pp. 1642–1645. DOI: 10.1126/science.1127344 (cit. on p. 4).
- [17] Samuel T. Hess, Thanu P. K. Girirajan, and Michael D. Mason. “Ultra-High Resolution Imaging by Fluorescence Photoactivation Localization Microscopy”. *Biophysical Journal* 91.11 (Dec. 2006), pp. 4258–4272. DOI: 10.1529/biophysj.106.091116 (cit. on p. 4).
- [18] Michael J. Rust, Mark Bates, and Xiaowei Zhuang. “Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)”. *Nature Methods* 3.10 (Oct. 2006), pp. 793–796. DOI: 10.1038/nmeth929 (cit. on p. 4).
- [19] Roy Wollman and Nico Stuurman. “High throughput microscopy: from raw images to discoveries”. *Journal of Cell Science* 120.21 (Nov. 2007), pp. 3715–3722. DOI: 10.1242/jcs.013623 (cit. on p. 4).
- [20] Emmanuel G. Reynaud, Jan Peychl, Jan Huiskens, and Pavel Tomancak. “Guide to light-sheet microscopy for adventurous biologists”. *Nature Methods* 12.1 (Jan. 2015), pp. 30–34. DOI: 10.1038/nmeth.3222 (cit. on p. 4).

- [21] Jeffrey M. Perkel. “The struggle with image glut”. *Nature* 533.7601 (Apr. 2016), pp. 131–132. DOI: 10.1038/533131a (cit. on p. 4).