NOBEL PRIZE WINNERS

Eric Betzig's Lattice Light Sheet Microscopy

TOPTICA

Pushing the Limits of Science

INTRODUCTION

Have you ever wondered what to do after you received a Nobel Prize? In 2014, Eric Betzig from the Janelia Research Campus in Ashburn, Virgina was in the middle of a discussion on microscopy lasers with Frank Lison from TOPTICA, when he got a phone call. It was the Nobel committee. And they informed him of his shared Nobel Prize award in chemistry for his contribution on super-resolution fluorescence microscopy.

At this time, he was working at a new microscopy technology that he thinks could have an even greater impact than his Nobel Prize-awarded Photoactivated Localization Microscopy (PALM).

Only weeks after the Nobel committee's call, his paper on "Lattice Light Sheet Microscopy" was published in Science magazine and demonstrated how to take microscopy to the next level.

Since the invention of the microscope, we have learned a lot about biological processes. But the more details we find, the more we become aware of what is still to be discovered. By applying coherent light sources like lasers many new possibilities have emerged to improve resolution in microscopy and even to beat the limit of diffraction. Also Eric Betzig aimed at pushing these limits in microscopy.

With PALM, for which he received the Nobel Prize, he developed a technique to resolve two neighboring fluorophores with a distance below the diffraction limit. His trick: He used a photoactivatable fluorescent protein for labeling, which can be stochastically activated with pulsed laser light of a certain wavelength. This way it is unlikely to excite two neighboring fluorophores at the same time. And after multiple acquisitions, an image reconstruction software calculates the final image from the time-series of fluorescent partial images. But for most real-time observations PALM is too slow. And according to a Washington Post article Betzig said: "I'd been looking at those pictures my whole life. It was time to take a look at the living stuff in action. [1]"

But how to achieve such a high temporal and spatial resolution necessary for this purpose? To watch cells and their development or other biological entities in detail, microscopic techniques had to evolve again. Stefan Hell, the other Nobel laureate of that year, is a name forever connected to such contributions [2].

LIGHT SHEET MICROSCOPY

Commonly used imaging techniques like widefield and confocal fluorescence microscopy suffer some severe drawbacks. Usually, the light source illuminates the entire thickness of the probe. But the image acquisition itself takes place only in the focal plane.

This approach causes unnecessary damage to the probe and unwanted photobleaching of the fluorophores in the entire sample, not just the region of interest [3]. So why not restricting the illumination to a single plane? This is the idea of light sheet microscopy (LSM). In standard LSM, a laser beam with a Gaussian intensity distribution is used for generating a light sheet. Therefore, it is confined to the focal plane with a cylindrical lens that is placed perpendicular to the direction of observation.

But as a gaussian beam is still divergent, focusing it to its minimum thickness results in a light sheet with either a too small field of view or with severe out-of-focus illumination [4].

Thus, a Gaussian beam is not the best choice for light sheet illumination with sub-cellular resolution.

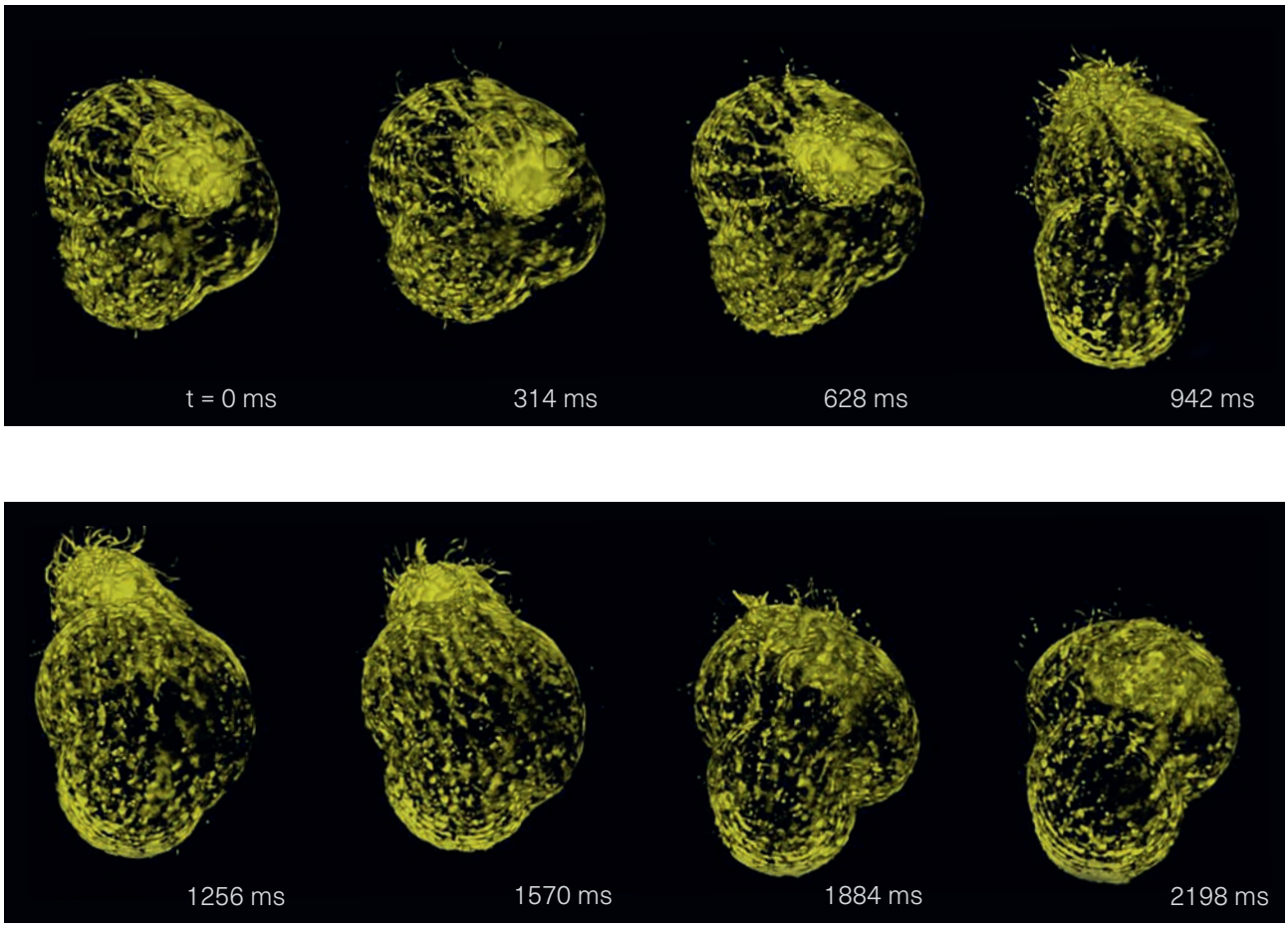

Figure 1: Volume renderings at eight consecutive time points of a single specimen of the protozoan T. thermophila to visualize intracellular dynamics in three dimensions (Bi-Chang Chen et al., Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution", SCIENCE 24 Oct 2014, Vol 346, Issue 6208, DOI: 10.1126/science.1257998. Reprinted with permission from AAAS.).

BESSEL BEAM PLANE ILLUMINATION

But there are alternatives to a Gaussian beam that were also explored by Eric Betzig and his co-workers – Bessel beams [4].

Bessel beams are waves whose amplitudes are described by a first-order Bessel function and they offer very interesting optical properties: ideal Bessel beams are non-diffractive i.e. they do not spread out as they propagate. And they are even "self-healing" by reforming after being partially obstructed. Such beams are generated from a Gaussian beam by confining it to an annulus in the rear focal plane. The extent of its central beam at the sample can be predefined by the thickness of the annular beam in rear focal plane without substantially increasing its divergence.

When sweeping such a Bessel beam along one axis, a light sheet with a narrower core can be realized (see figure 2). This technique allows to create much thinner light sheets compared to Gaussian beam LSM [3]. But still, it has side bands resulting from the side lobes of the Bessel beam that broaden the illumination beyond the focal plane.

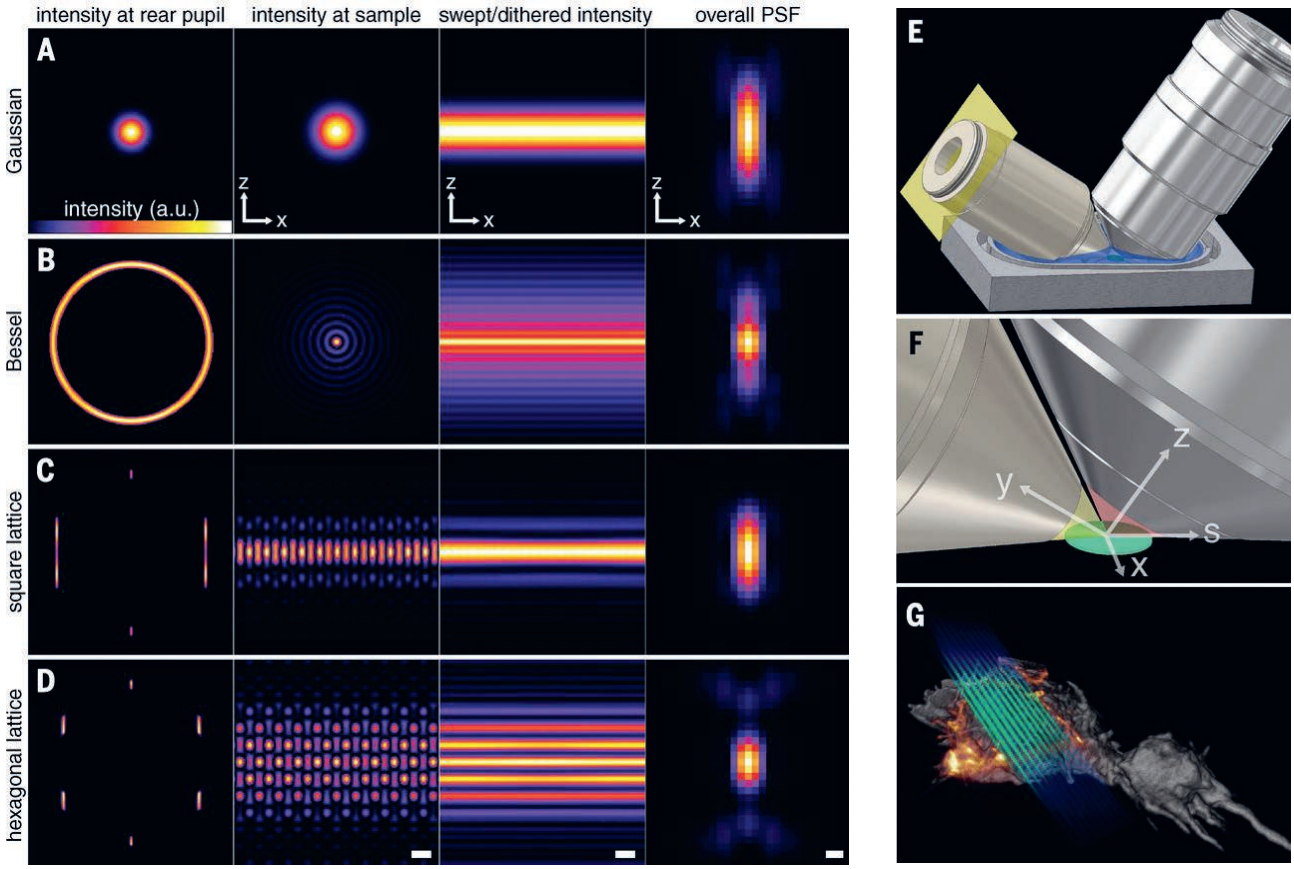

Figure 2: Comparison between methods of light-sheet microscopy as given by Bi-Chang Chen et al., Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution", SCIENCE 24 Oct 2014, Vol 346, Issue 6208, DOI: 10.1126/science.1257998. Reprinted with permission from AAAS.

LATTICE LIGHT SHEET MICROSCOPY

To reduce the side bands of the light sheet Betzig and his team combined the Bessel light sheets with a structured illumination microscopy (SIM) approach that is usually implemented in widefield applications [5].

In structured illumination microscopy two or more coherent light beams are generated by a grating or diffracto-optical element. Interference of the beams within the probe results in a spatially structured illumination pattern with pattern sizes below the limit of diffraction. Using such illumination patterns allows for high-resolution and even super-resolution images by reconstructing the images from image series with appropriate algorithms.

To create an optical lattice, the laser beams are spatially modulated via a fast-switching spatial light modulator (SLM). The SLM is placed before an annular mask that subsequently generates an optical lattice made of Bessel beams [3]. In general, these lattice light sheets can be operated in two different modes: One could either use the lattice for SIM putting the emphasis on maximal spatial resolution. Or one could optically move the lattice structure creating an optimized light sheet and offering maximal temporal resolution while still operating in the range of the diffraction limit [3].

For 3D Lattice Light Sheet Microscopy, the sample is moved through the light sheet and 2D images are recorded in the meantime. The images are combined to a 3D image and the process is repeated to create movies on the cellular level.

When the Betzig team implemented an array of seven independent Bessel beams to improve the temporal resolution, they also found that spreading the power to many foci substantially reduced the phototoxicity compared to a single beam with the same power - a major issue when dealing with living biological systems.

LATTICE LIGHT SHEET MICROSCOPY BECOMES “OPEN SCIENCE”

With Lattice Light Sheet Microscopy, Eric Betzig and his team set a new standard in microscopy. But the best technology won’t help if it stays hidden in the lab. Here, the Janelia Research Lab makes a remarkable offer: Under an Open Source license agreement, people around the world are encouraged to rebuild the microscope developed by Eric Betzig. The support includes data, software and information. And they partner with suppliers of components recommended for the setup. This way Janelia can ensure that interested users receive suitable components for reasonable prices and that the technology’s reach is maximized.

One especially important component of the setup is the laser source. It is easy to see that for the Bessel Beam Plane Illumination or other evolving microscopy techniques, one key is the availability of stable laser sources with single-mode beam quality. Because only they allow to use all the particularities of light like creating diffraction patterns for structured illumination or generating Bessel beams of high quality. And exactly these unique properties of the laser allowed Eric Betzig to circumvent the limit of diffraction and pave the way for high-end real-time cell imaging.

So, what to do after a Nobel Prize and transforming microscopy a second time? „The eventual goal is to marry all of my work together”, Eric Betzig revealed to the Washington Post [2]. “To make a high-speed, high-resolution, low-impact tool that can look deep inside biological systems.”

REFERENCES

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[4] Planchon, Thomas A., et al. „Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination.“ Nature methods 8.5 (2011): 417-423.

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TOPTICA LASERS FOR THE OPEN SOURCE KIT OF ERIC BETZIG'S MICROSCOPE DESIGN

The high-performance single mode diode lasers iBeam smart at 405 nm and 445 nm are key components for the Janelia Research Lab Open Source technology offering. It allows people around the world to rebuild the microscope developed by Eric Betzig and maximize that technology's reach.

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