Microscopy TODAY

Coming Events

2015

Quantitative BioImaging Conference
January 7–9, 2015
Institute Pasteur, Paris, France
www.quantitativebioimaging.com

PITTCON 2015
March 8–12, 2015
New Orleans, LA
www.pittcon.org

American Chemical Society
March 22–26, 2015
Denver, CO
www.acs.org

Focus on Microscopy
March 29–April 1, 2015
Göttingen, Germany
www.focusonmicroscopy.org

Novel Techniques in Microscopy
April 12–15, 2015
Vancouver, Canada
www.osa.org/en-us/meetings/optics_and_photonics_congresses/optics_in_the_life_sciences/novel_techniques_in_microscopy

MSC-SMC Annual Meeting
May 26–29, 2015
Hamilton, Ontario, Canada
www.bimr.ca/events/msc-smc-annual-meeting-2015

Microscience Microscopy Conference
June 29–July 2, 2015
Manchester, United Kingdom
www.mmc2015.org.uk

Microscopy & Microanalysis 2015
August 2–6, 2015
Portland, OR
www.microscopy.org

2016

Microscopy & Microanalysis 2016
July 24–28, 2016
Columbus, OH
www.microscopy.org

2017

Microscopy & Microanalysis 2017
July 23–27, 2017
St. Louis, MO
www.microscopy.org

2018

Microscopy & Microanalysis 2018
August 5–9, 2018
Baltimore, MD
www.microscopy.org

2019

Microscopy & Microanalysis 2019
August 4–8, 2019
Portland, OR
www.microscopy.org

More Meetings and Courses
Check the complete calendar near the back of this magazine.

Carmichael's Concise Review

Better Resolution in Space and Time

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Biologic processes occur in four dimensions and can be too small, too fragile, and/or too fast to observe with available microscopes. A multi-disciplinary team of 27 scientists headed by 2014 Nobel laureate Eric Betzig, which included Bi-Chang Chen, Wesley Legant, and Kai Wang, have invented a new microscope that yields better resolution in space and time, and also keeps phototoxicity to a minimum [1]. The key to this extraordinary increase in spatiotemporal resolution was the creation of an ultrathin, non-diffracting light sheet of low intensity that could be spread over a broad area. This is a 2D optical lattice that is created by the coherent superposition of a finite number of plane waves traveling in a certain well-defined direction. Ideally it propagates indefinitely in a direction y without changing its cross-sectional profile, which extends infinitely in x and z. In practice, this is accomplished by confining the illumination at the rear pupil plane of the excitation objective with an annulus of finite thickness to confine the pattern in the z plane to produce a sheet rather than a block of light. This does limit some of the advantages of an ideal lattice sheet, but Chen et al. have identified several 2D optical lattices that, over a sufficient field of view, can be optimized to either confine the excitation

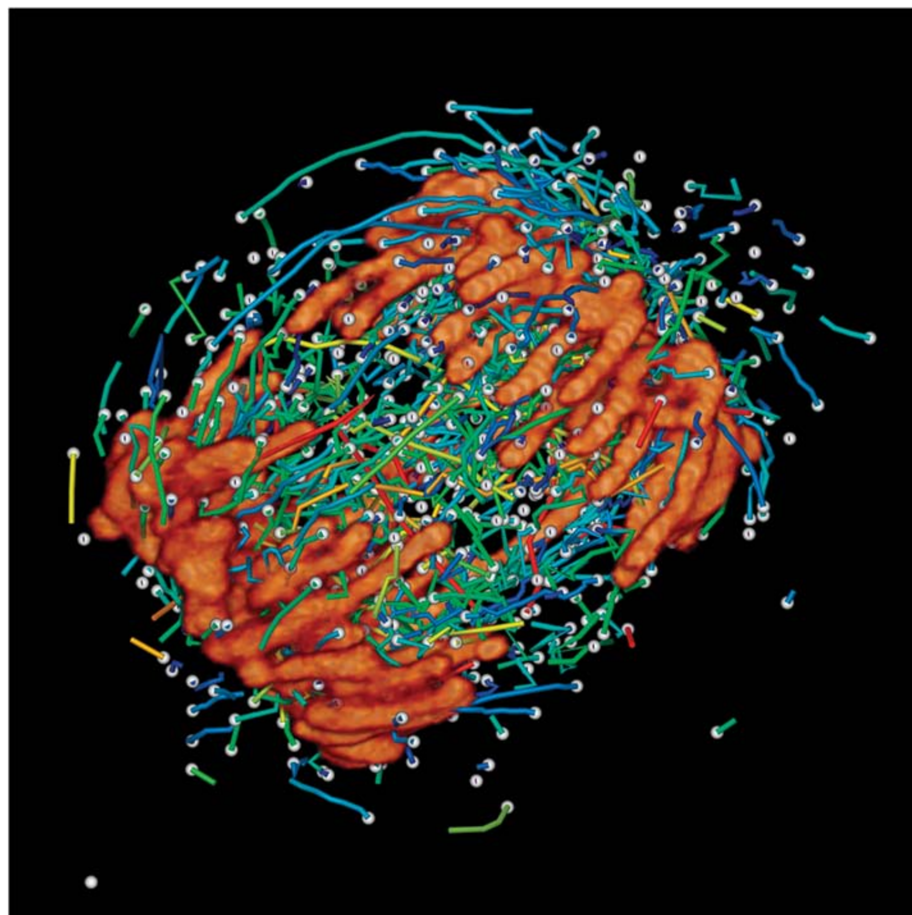

Figure 1: Chromosomes (orange) surrounded by tracks denoting the ends of growing microtubules marked with the protein EB1, at one time point out of 500 in a movie of a dividing HeLa cell. The movie is Movie 5 at http://vimeo.com/album/3098015. Tracks are color-coded according to the microtubule growth rates. The color code is from zero (purple) to 1.2 microns/sec (red) in standard rainbow order. The image volume is 32 × 32 × 20 microns. Credit: Betzig Lab, Janelia Research Campus / HHMI; Yuko Mimori Kiyosue, Riken CDB

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tightly to the *xy* plane, minimize the *z* resolution, and define the overall point spread function of the microscope; or provide super-resolution in the *xz* plane by structured illumination microscopy (SIM). There are many more technical details, but, in brief, the specimen is moved through an ultrathin light sheet, and the fluorescence that is generated is recorded as a series of 2D images. These are assembled into a 3D image, and the process can be repeated to build a 4D data set of cellular dynamics.

Chen et al. operated the microscope in one of two modes: (1) a super-resolution SIM mode that could be reconstructed as a 3D image with resolution beyond the diffraction limit, or (2) a high-speed dithered mode that produced a 4D data set that was diffraction-limited. This latter mode yielded high-resolution, high-contrast 2D imaging within multicellular specimens at up to 100 frames per second for thousands of time points! With certain adjustments, the dithered mode can achieve a resolution of 230 nm in the *x* axis and about 370 nm in the *z* axis for green fluorescent protein excitation and emission. This is only slightly worse spatial resolution than the SIM mode, but the temporal resolution is more than seven times greater. Furthermore, the dithered mode can image many specimens indefinitely in 3D without photobleaching or noticeable phototoxicity. This includes even the notoriously light-sensitive slime mold amoeba *Dictyostelium discoideum*. Chen et al. found that unless the additional resolution of the SIM is absolutely essential, the dithered lattice light sheet is the preferred option for most biological systems.

Chen et al. demonstrated the usefulness of their microscope on 20 different biological processes spanning four orders of magnitude in space and time. This went from imaging binding kinetics of single molecules to subcellular protein localization and dynamics during embryogenesis in the transparent roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Among the many examples studied, the dynamic instability of microtubules was seen in 3D, including microtubule tip positions, growth phase lifetimes, and growth velocities across an entire HeLa cell during mitosis (Figure 1). For processes that evolve slowly compared to the speed of this microscope, extra bandwidth can be expended in a fifth dimension by adding more wavelengths and imaging in more colors. It may prove possible to study the 4D interplay of a dozen or more proteins in the same cell at the same time and to understand how they orchestrate complex cellular functions. This is truly an amazing microscope that a qualified microscopist can apply to use [3]!

References

[1] B.-C. Chen et al., *Science* 346 (2014) 1257998, DOI: 10.1126/science.1257998.

[2] The author thanks Dr. Eric Betzig for reviewing this article prior to publication.

[3] Researchers can apply to access the lattice light sheet microscope as visitors through the Advanced Imaging Center at Janelia Farm (www.janelia.org/aic), a research campus of the Howard Hughes Medical Institute (HHMI) in northern Virginia. Documentation for a construction of a copy of the microscope is available after execution of a research license with HHMI. Portions of the technology of this microscope are protected by a U.S. Patent.

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TKD IPF map of strained Cu. Step size 4 nm. Courtesy: Saritha Samudrala, Kevin Hemker and Pat Trimby.

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