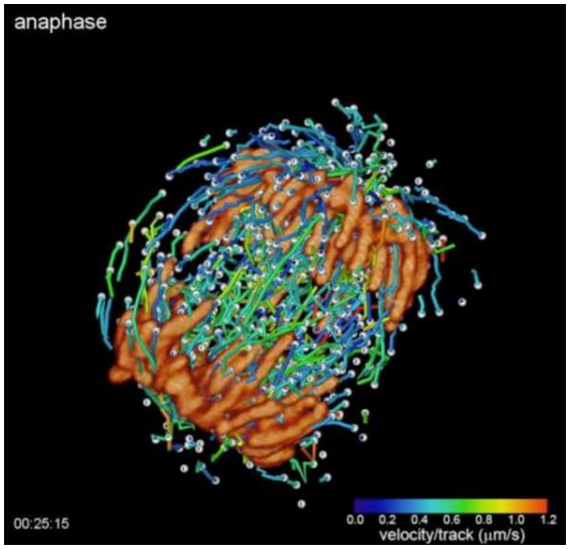
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Cell division as seen through lattice light-sheet microscopy

December 5, 2014–In 1873, Ernst Abbe, the father of modern microscopy, published a formula demonstrating that the resolution limit of a light microscope is limited by the wavelength of light, and that it is not possible to obtain a better resolution than half the wavelength of light—and in fact, this remained true for over a century. But as undeterred scientists continued their quest to visualize samples in more detail and at greater depths, the accumulation of their efforts has brought us to an age where the observation and tracking of a single molecule inside a cell is now possible. The impact and importance of these developments and advances in so-called superresolution microscopy for science was recognized by this year's Nobel Prize in Chemistry being awarded to the three pioneers of this technology.

One of this year's Nobel Laureates, Eric Betzig (Howard Hughes Medical Institute: HHMI), and his collaborators have unveiled a new superresolution microscope system they call 'lattice light-sheet microscopy,' which has now been reported in the journal *Science*. They also demonstrated the microscope's capabilities for use in biological applications using several model samples ranging in different scales of magnitude, including a cell line expressing a fluorescent marker for a microtubule plus end marker that was generated by former RIKEN CDB Unit Leader, Yuko Mimori-Kiyosue (currently Unit Leader, RIKEN CLST Cellular Dynamics Analysis Unit), one of the paper's co-authors. Analysis of the imaging data obtained using their microscope revealed surprising new insights about the microtubule dynamics in cells undergoing cell division.



Observing behavior of chromosomes (orange) and microtubule plus-end marker EB-1 during cell division in a fluorescently labeled HeLa cell using the lattice-light sheet microscope. Trajectory of EB-1 is illustrated by colored lines, which indicate velocity of elongation.

Besides the image resolution of a microscope, the speed at which a microscope can capture images is another important factor to consider when attempting to visualize intracellular structures and the rapid movement of molecules in three dimension (3D). While the advent of superresolution microscopy has greatly improved image resolution, carrying out 3D live imaging of living organisms with this system remained a challenge because the lengthy time required to scan a sample was phototoxic for living samples.

In their new microscope system, the Betzig group made several important modifications to the currently available light-sheet microscope, which is considerably less invasive than the widefield and confocal microscopes more commonly used for live imaging. They had previously adopted a method to illuminate samples using an ultrathin "sheet" of light produced by a linear array of non-diffracting Bessel beams, which produced a better contrasting image by restricting the irradiated area and by greatly reducing the resolution degradation along the z-axis. This strategy was likewise effective in illuminating a broader area across a single plane, minimizing the time required to capture images of a sample. However, resolution and sample scan speed of the Bessel beam method was insufficient to

visualize movement of fine subcellular structures. The group then went a step further to develop a system that would generate light sheets that were created from a two-dimensional optical lattice, lattice light sheet. This allowed them to capture more detailed 3D images of samples at higher speeds, with less damage to cells, and over longer intervals compared to existing live-imaging systems.

Betzig and colleagues highlight the improvements of their lattice-light microscope over other live imaging techniques by presenting data from their observations of different biological processes such as embryonic development in *C. elegans* and *Drosophila*, and T-cell behavior. They were also successful in imaging the process of cell division in a fluorescently labeled cell line generated by Mimori-Kiyosue to reveal the dynamic movements of microtubule elongation and of each chromosome at an unprecedented level of detail. The higher speed of the lattice-light microscope to scan a cell and the higher image resolution allowed the group to observe even the microtubule dynamics taking place inside the mitotic apparatus. Preliminary analysis of the imaging data by Mimori-Kiyosue suggests that the mitotic apparatus functions in a manner different to what was previously thought. This and other findings presented hint that previously unknown mechanisms may be unveiled by using this technology.

“The capacity of this new microscope to analyze samples at cellular and subcellular levels will have tremendous impact on our understanding of not only cell division, but also the life science field as a whole,” explains Mimori-Kiyosue. “I will continue my efforts to bring this new microscope technology to Japan.”