Notes on
Observing the cell in its native state: imaging subcellular dynamics in multicellular organisms (Betzig et al.)

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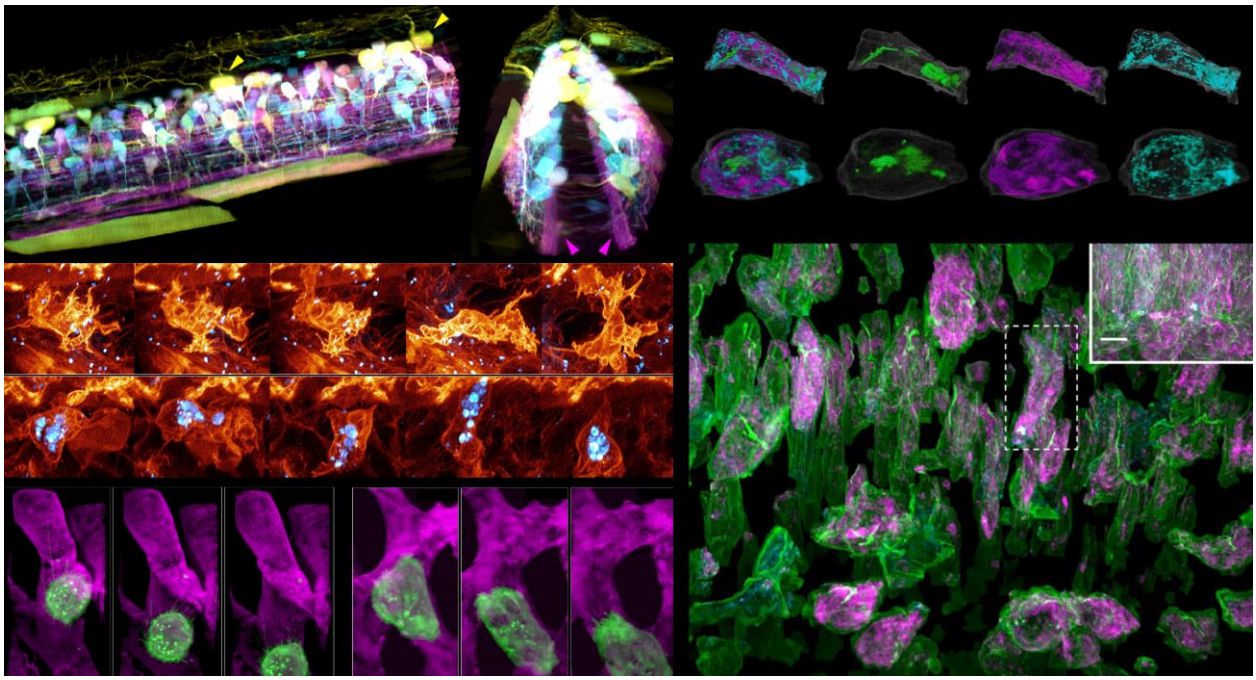
Overview

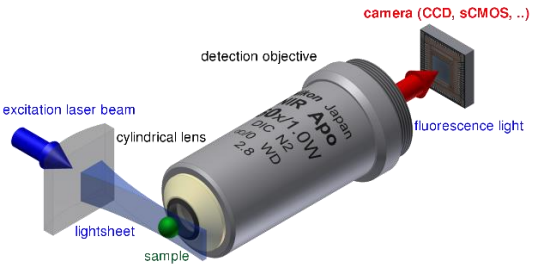
- Eric Betzig, a 2014 Nobel laureate in chemistry (for playing a key role in developing super-resolution fluorescence microscopy), has continued to advance biological imaging technologies.
- In their 2018 paper, "*Observing the cell in its native state: imaging subcellular dynamics in multicellular organisms*," Betzig's group combined lattice light sheet microscopy and adaptive optics to acquire 3-dimensional images and videos of physiological processes in vivo with subcellular resolution, achieving unprecedented detail and clarity.



Source: (Liu et al., 2018)

Light sheet microscopy

- Light sheet microscopy uses a laser and a cylindrical lens to project a plane of illumination through a sample.
- As the sheet only illuminates a single plane of the sample at a time, this technique decreases photodamage relative to other methods which pass light through the entire sample at once. In addition, the single plane illumination decreases background noise and so generates images with high contrast.
- Light sheet microscopy operates rapidly since it scans entire layers of the sample all at once rather than scanning one point of light at a time (the latter is common with other types of microscopy).

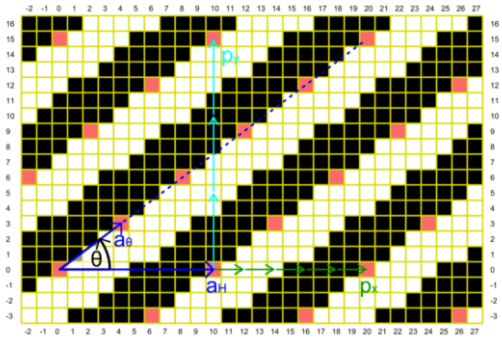


Source: Jan Krieger, CC BY-SA 3.0, commons.wikimedia.org

- Each layer of the sample is imaged in this way before the layers are stacked to reconstruct a 3-dimensional image. Betzig's group used a mathematical algorithm called deconvolution to remove out-of-focus light and enhance more focused light within the Z-stacks generated by his lattice light sheet microscopy technique.

Lattice light sheet microscopy

- In lattice light sheet microscopy, the input laser is first stretched (in the x direction) by a pair of cylindrical lenses and then compressed (in the z direction) into a sheet by another pair of cylindrical lenses oriented perpendicularly to the first pair.
- Lattice light sheet microscopes combine Bessel beams with 2D optical lattices using an optical element called a spatial light modulator. The spatial light modulator uses a ferroelectric liquid crystal display to create programmable gratings that diffract incoming light into a customized pattern. This is displayed in the figure from Förster et al., where a spatial light modulator displays a customized grid of white squares (light can pass through) and black squares (light is blocked) which forms a diffraction grating.



Source: (Förster et al., 2014)

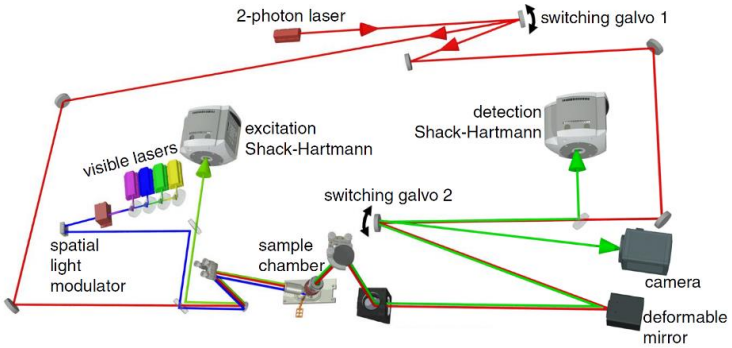
    - Bessel beams are fields of electromagnetic radiation which can be mathematically described by Bessel functions of the first kind. Unlike most electromagnetic radiation, Bessel beams do not spread out as they propagate. As such, they do not form diffraction patterns. Although ideal Bessel beams are physically impossible, but approximate forms of the phenomenon can be created.
    - 2D optical lattices arise from the interference of beams of light that exhibit periodic behavior in two dimensions. They can form the same set of

patterns found in 2D Bravais lattices (which are a mathematical formulation for crystal structures).

- On their own, neither Bessel beams nor 2D optical lattices are useful for light sheet microscopy, but they achieve superior properties for imaging when properly implemented together.
    - Bessel beams contain the same amount of energy in their “side lobes” as they do in their central peaks. For this region, they cause excessive illumination outside of the targeted plane. This is problematic for light sheet microscopy since the technique depends on having a focused plane of light.
    - Despite their name, 2D optical lattices extend into 3D space (since many “copies” of the planar lattice occur along the z direction). This is similarly problematic for light sheet microscopy since the technique depends on the light exhibiting confinement to the xy plane of focus.
    - These issues can be overcome by combining Bessel beams and 2D optical lattices. To achieve this, a ring (or annulus) of illumination is used to destructively interfere with the side lobes of the Bessel beams, creating Bessel-Gauss beams. Then an array of coherent Bessel-Gauss beams is generated (two waves with a constant phase difference, the same frequency, and the same waveform are coherent). This array of Bessel-Gauss beams is suitable as a light sheet for lattice light sheet microscopy.

Adaptive optics

- The light used for excitation in lattice light sheet microscopy traverses different regions of the sample relative to the detected light. As such, the light involved in excitation and the light involved in detection are subject to different aberrations.



Source: (Liu et al., 2018)

- To adjust for such aberrations, Betzig’s group used a two-photon excitation beam from an ultrafast Ti:Sapphire laser. This beam creates a “guide star” (this term comes from a similar technique used in astronomy) which acts as a reference. The guide star is scanned over the entire focal plane so as to compute an average correction since average correction has more accuracy than a correction from a single point in the sample.
- Next, a switching galvanometer SG1 (a device which rotates a mirror back and forth) facilitates transfer of the two-photon excitation beam to either the excitation or detection arms of the microscope as needed.

- For the detection beam, the light generated from the scanned guide star is collected and sent to a device called a Shack-Hartmann wavefront sensor using another switching galvanometer SG2.
    - The Shack-Hartmann wavefront sensor contains an array of small sensors which measure the “tilts” of the incoming plane waves. By measuring the local tilt of each small wavefront composing the detected light beam, the overall shape of any optical aberration can be approximated.
    - Then a deformable mirror is modified to precisely compensate for the aberration measured by the Shack-Hartmann wavefront sensor. The second switching galvanometer SG2 transfers the light to this deformable mirror. After reflecting from the deformable mirror, the aberration is corrected and the detection objective collects the light to create an image.
- For the excitation beam, the light from the two-photon excitation laser is scanned over the sample as a guide star and then collected by the excitation objective.
    - The collected light is transferred to another Shack-Hartmann wavefront sensor. Once again, the sensor measures an approximate representation of any optical aberration which occurs.
    - Next, the spatial light modulator used in creating the light sheet itself applies the appropriate correction to the excitation beam. This is highly effective since the spatial light modulator provides exquisite control over the output light sheet.

References

Chen, B.-C., Legant, W. R., Wang, K., Shao, L., Milkie, D. E., Davidson, M. W., … Betzig, E. (2014). Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution. *Science*, *346*(6208). Retrieved from http://science.sciencemag.org/content/346/6208/1257998.abstract

Förster, R., Lu-Walther, H.-W., Jost, A., Kielhorn, M., Wicker, K., & Heintzmann, R. (2014). Simple structured illumination microscope setup with high acquisition speed by using a spatial light modulator. *Optics Express*, *22*(17), 20663–20677. http://doi.org/10.1364/OE.22.020663

Liu, T.-L., Upadhyayula, S., Milkie, D. E., Singh, V., Wang, K., Swinburne, I. A., … Betzig, E. (2018). Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. *Science*, *360*(6386). Retrieved from http://science.sciencemag.org/content/360/6386/eaaq1392.abstract