Lattice Light Sheet Microscopy: High Resolution 4D Visualisation of Living Cellular Processes

Abstract

Lattice Light Sheet Microscopy (LLSM) is a fluorescence imaging technique which enables visualisation of cellular processes in living biological samples at high spatio-temporal resolution and rapid sampling rates. By scanning through the volume of the sample using extremely thin planes of low-intensity laser light, the impact of phototoxicity and photobleaching on cells is greatly reduced. The Laser Quantum gem series of cw fibre-coupled lasers, which offer excellent beam quality and exceptional long-term power stability, are ideal candidates of laser sources for LLSM.

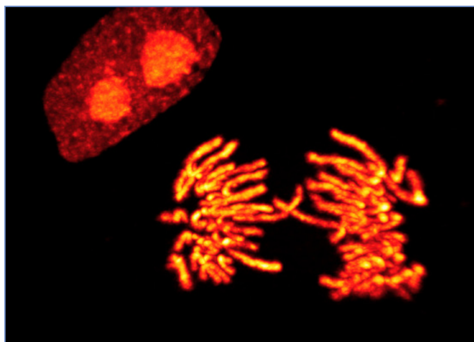

Figure 1: Credit Gary Gorbsky, Oklahoma Medical Research Foundation

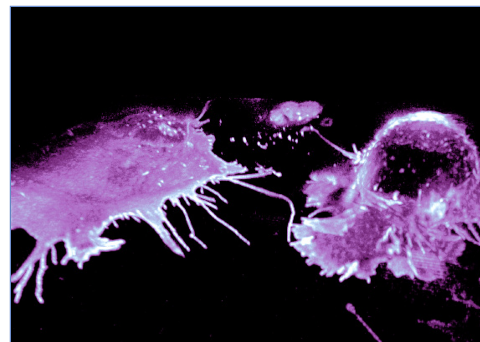

Figure 3: Credit Ema Cocucci, Ohio State University

Lattice light sheet microscopy, a novel combination of techniques drawn from light sheet microscopy, Bessel beam and super-resolution microscopy, was developed by Professor Eric Betzig and colleagues at the Howard Hughes Medical Institute Janelia Research Campus[1].

It was first described[2] in 2014, soon after Prof Betzig had been jointly awarded that year's Nobel Prize for Chemistry, with Prof Stefan Hell and Prof William Moerner, "for the development of super-resolved fluorescence microscopy".

Optical lattice light-sheets used in LLSM can be much thinner than those formed from Gaussian beams, and they have much lower background signal than Bessel beams, both of which have been used in conventional light sheet microscopy. Such extremely thin light-sheets offer potential for significant advantages in signal-to-noise and sensitivity, and substantial reductions in phototoxicity and photobleaching in live-cell microscopy.

Fluorescence microscopy is an invaluable tool for imaging biological samples, but presents unique challenges for the detailed study of delicate living structures and processes. The goal is to achieve higher levels of resolution at faster sampling rates without causing photodamage or bleaching to the specimen.

In conventional widefield or confocal microscopes, the full three-dimensional depth of the specimen is illuminated but in-focus fluorescence is detected only from a single two-dimensional plane. This makes such systems highly inefficient for 3D imaging of living specimens.

In light sheet microscopy (LSM), the sample is illuminated by scanning sheets, or planes of laser light, through its volume. By coupling a separate excitation lens in a perpendicular axis to the detection lens, the excitation of the specimen is confined to the volume under observation. This enables much faster acquisition speed whilst at the same time significantly reducing effects of photoxicity and photobleaching.

In LSM systems which employ a Gaussian profile beam however, optical resolution is limited by the effects of diffraction. Non-diffracting Bessel beams can also be used, but there can be as much energy in the side-lobes of the Bessel function as the central spot, leading to illumination out of the depth of field.

In LLSM, the plane of illumination is created by a two-dimensional lattice of Bessel beams, controlled and maintained using a spatial light modulator (SLM) which allows pixel-level control of the wavefront.

By carefully selecting and tuning the period of the lattice, energy in the side-lobes can be reduced by destructive interference. Unwanted frequencies introduced in this process are removed by filtering with an annulus positioned in the back focal plane of the objective. The resulting ultrathin light-sheets offer improved axial resolution over conventional Bessel beam light sheet microscopy, with little or no photobleaching or background outside of the focal plane. These 2D optical lattices are scanned through the specimen plane-by-plane to build up a 3D image. Imaging speeds of hundreds of planes per second are possible because the entire field of view is illuminated simultaneously.

Suitable source lasers for LLSM are required across a range of visible wavelengths for excitation of different fluorophores. They must offer very good beam quality and highly stable output power over long and short time scales. The Laser Quantum **gem** series of solid-state cw fibre-coupled lasers offer excellent beam spatial profile (M^2 <1.1), beam pointing stability (<10 µRad/°C) and output power stability specifications (<0.8% RMS over 100 hours at constant temperature) in an exceptionally robust, compact package.

LLSM is not without limitations, including some restrictions on sample mounting. As the detection system is positioned orthogonal to the plane of excitation, free space access to the sample from above must be available.

Another issue with LLSM is depth of penetration, which can be limited by aberrations and distortions introduced by the tissue itself as light passes deeper.

However, new developments recently announced by Prof. Betzig and colleagues[3] offer potential for improved resolution at greater depths in tissue by utilising adaptive optics to introduce corrections to the optical train (AO). In AO-LLSM, a reference beam, or "guide-star" from within the sample provides a measure of the aberrations being introduced, which can then be subtracted in real-time using an SLM, to sharpen the final image. Utilising these techniques, researchers report capturing hours of video of endocytosis, organellar dynamics, and neurite growth in developing zebrafish embryos.[4] Such approaches add complexity but offer the promise of extending the gains of LLSM to processes taking place even deeper in living tissue.

LLSM has opened a new realm of high resolution 4D imaging of living cells and processes, with unprecedented levels of detail now available to researchers. Further developments in the technique promise to extend its capabilities into new domains. An essential component of such systems will continue to be reliable and highly stable laser sources, and Laser Quantum's **gem** series offers design and performance advantages.

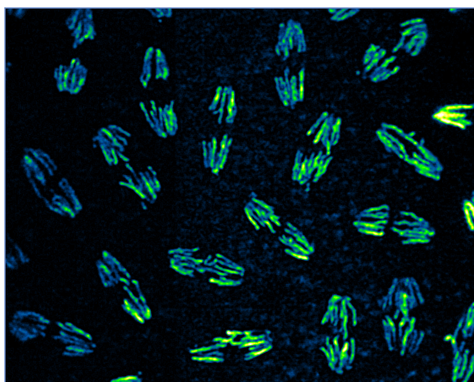

Figure 2: Credit Edouard Bertrand, Institut de Genetique Moleculaire de Montpellier

Figure 4: Laser Quantum **gem** laser used to excite fluorophores

Acknowledgements: Images courtesy of the Advanced Imaging Center, Howard Hughes Medical Institute Janelia Research Campus. These are supplied for information purposes only, and do not imply endorsement by the AIC or HHMI Janelia of Laser Quantum lasers.

References: (1). Betzig Lab https://www.janelia.org/lab/betzig-lab
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