Light-sheet microscopy used for tracking particles

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Abstract

Fluorescence microscopy is one of the cornerstones of modern biology but has generally been limited to 2D culture dishes. Light-sheet microscopy, a recent advance which was awarded Nature Method of the Year in 2014, allows fast, non-invasive 3D imaging across an entire organism. This works by decoupling illumination and detection such that the microscope only illuminates a thin section of tissue at a time. By scanning this *light-sheet* through an organism we can image in 3D, more quickly and with less damage than other techniques such as confocal microscopy. In this work, a custom digitally scanned light-sheet microscope was built, for which the technology was applied and developed to enable two biological studies: the study of material properties of developing embryos and the tracking of virus particles in live cells.

In addition to designing and constructing a light-sheet fluorescence microscope, several technological improvements were also investigated to better address for these biological questions. The first was a three-dimensional region-of-interest technique which greatly simplifies volumetric imaging calibration whilst also being more robust, with an observed $42\,\%$ improvement in light collection efficiency compared to current approaches. The projective mathematical theory, used in this technique, was then applied to optical projection tomography to produce a new triangulation-based reconstruction algorithm that is robust to affine sample motion, including mechanical jitter and systematic drift. The second improvement for light-sheet microscopy builds upon confocal slit scanning, a technique used to increase image contrast whilst doubling the acquisition time for a single image. By exploiting the acquisition procedure for confocal slit scanning, full speed imaging with the same increased contrast was realised. Finally an open-hardware solution for multi-scale sample mounting was produced. These improvements to speed, contrast and acquisition speed in the light-sheet microscope allowed us to address the biological questions of interest.

First, the light-sheet microscope was applied to the tracking of virus particles (virions) in live cells. These particles invade host cells and hijack their machinery to replicate and then spread causing disease. To investigate viral infection, the pathways in which virus particles travel need to be visualised *in vivo* and in three dimensions. Light-sheet microscopy is better suited than other techniques for tracking virus trafficking in 3D as virions are exceptionally small and fast-moving. In this work Herpes Simplex Virus 1 was considered. HSV-1 is the widespread cause of cold sores and genital herpes. Furthermore, it serves as a biological model for other Herpesviruses which are associated with many serious diseases including chickenpox and certain lymphomas.

Then, the tracking of particles in three dimensions was brought from the nanoscopic (tracking single virions) to the microscopic (tracking a single magnetic beads) scale in developing zebrafish embryos. Internal stresses within tissues induce cellular migrations that can govern the resultant anatomy of the organism. A technique was developed to mechanically probe deep tissue using magnetism. By embedding a magnetic bead in a live embryo, non-invasive magnetic fields were used to move the bead within the developing tissue. Using the model fitting of bead trajectories in simple push-relax-pull-relax experiments, local mechanical properties were extracted. In addition, the mechanical roles of key proteins in embryonic development were inferred by comparing these extracted material properties between genetically modified and wild type embryos. For the first time, our investigations have contradicted previous reports that rho-kinase increases cell stiffness in embryonic development.