A PLATFORM FOR HIGH-THROUGHPUT SUPER-RESOLUTION MICROSCOPY

Andrew E.S. Barentine[1,2], Leo Balduf[1], Michael R. Grace[1], Yu Lin[1,2], Joerg Bewersdorf[1,2], David Baddeley[3,1]

1 Dept. of Cell Biology, Yale University, New Haven, CT, USA,
2 Dept. of Biomedical Engineering, Yale University, New Haven, CT, USA
3 Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand.

Email: d.baddeley@auckland.ac.nz

Single-molecule switching based microscopy techniques such as PALM and STORM allow impressive resolution improvements, but have traditionally been slow, limiting the types of applications which can be addressed. As these methods mature, there is a new focus on improving speed and throughput. The introduction of sCMOS cameras has allowed raw data to be acquired at significant speed (frame rates of over 800 frames/second, or a data rate of 800MB/s) but the processing of this data has been much slower – even with GPU acceleration. This is compounded by challenges in storing the significant volumes of data produced: when used continuously, an sCMOS camera will fill a 1TB PCIe SSD in around 20 minutes.

We have developed an open-source framework that allows both the analysis and storage of single-molecule localization data to be distributed over a small cluster of inexpensive machines and allows data to be analyzed in real time. Our software framework consists of: 1) a distributed file system optimized for imaging data, 2) efficient, real-time compression algorithms that reduce raw data volume by a factor of 5-10, 3) a task-distribution layer which allocates processing tasks to worker nodes in a way which takes advantage of data locality and 4) enhanced sCMOS-specific GPU-fitting algorithms.

When combined with a suitable, fully automated, microscope, this framework allows us to acquire 2-colour 3D super-resolved image-stacks of mammalian cell nuclei at a speed of roughly 3 cells/minute (~5000 cells/day) and to analyze the resulting data in real time. This opens up a wide range of new applications of super-resolution microscopy – from the generation of datasets which can be compared to population-based 'omics' techniques, to the investigation of rare events and interactions which only occur in a small fraction of cells.