The Octopus imaging cluster: a new facility for the LSF

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Introduction

The past year has seen major changes in the Lasers for Science Facility, in anticipation of the move of the group to the Research Complex at Harwell (RCaH) at the beginning of 2010. The LSF's operations in the biological imaging area have been expanded with the move of the Advanced Single Molecule Imaging and Dynamics (ASMID) facility previously located at Daresbury Laboratory. The ASMID facility specialized in multidimensional single molecule microscopy, and multicolour confocal microscopy, and is the ideal complement to the advanced multiphoton fluorescence lifetime imaging (FLIM) facility already operating in the LSF. The ASMID microscopes were moved from Daresbury at the beginning of 2009 (Fig. 1). Transporting the complex and delicate instrumentation was a major task, but it was achieved without incident (except for a tight squeeze through the R1 corridors!), and the microscopes are now operational in their temporary home, from where we will operate a full user science programme up to the move to RCaH. The Octopus cluster is the main facility offered by the Functional Biosystems Imaging Group of the LSF.

The Octopus cluster

Octopus is a new concept in large-scale imaging facilities, and consists of a central core of laser light sources, supplying illumination through optical fibres to a range of microscopy stations. The first generation of Octopus offers the following microscopy stations:

Figure 1. On a cold February morning, Marisa Martin-Fernandez, Chris Tynan, and Stephen Webb welcome the microscopes to RAL.

2 colour, 2 polarisation single molecule TIRF microscope. A laser source is coupled to a home-built objective-type Total Internal Reflection (TIRF) microscope via a single-mode fibre (Fig. 2). The excitation polarisation is purified using a Glan-Taylor polariser and then rotated between orthogonal polarisations using an achromatic liquid crystal polarisation rotator.

The fluorescence is split into orthogonal emission polarisations in each of two wavelength bands, giving four channels which are imaged onto the CCD simultaneously. Polarisation-sensitive dichroic mirrors are used to ensure that the fluorescence polarisation is

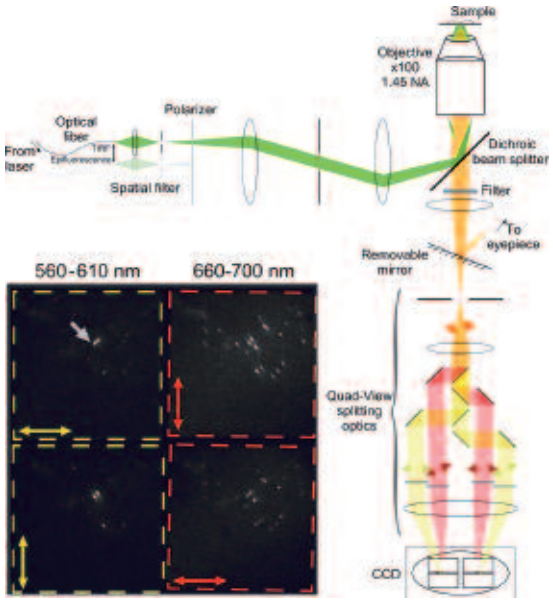

Figure 2. Experimental setup for multidimensional single molecule fluorescence imaging. The Quad-View optics splits the fluorescence into four spatially identical images on the CCD, which differ solely in their spectral and polarization properties. A typical image of single fluorescent molecules in the membrane of live cells is shown, in this case murine EGF-Cy3 and murine EGF-Cy5, which appear on the left and right halves of the image, respectively. (Double-headed arrows indicate the axis of fluorescence polarization.)

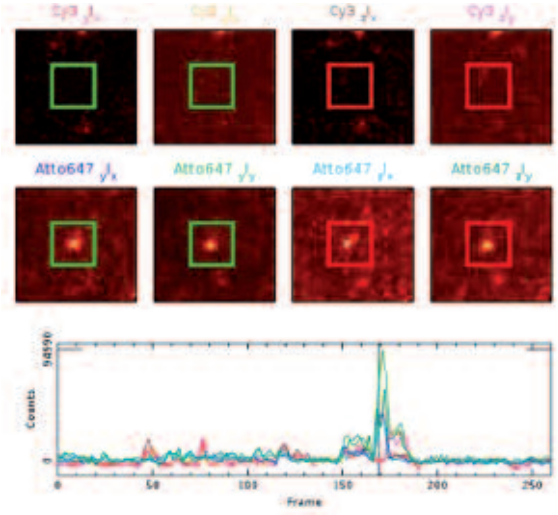

Figure 3. Multidimensional single molecule data from live cells labelled with EGF-Atto 647 and EGF-Cy3. Regions of interest extracted from the images, with all 8 channels are shown. The square superimposed on the image highlights a fluorescent spot detected and the intensity v time traces for this spot are shown below.

not affected. The switching of the rotator is synchronized to image acquisition such that alternate frames in a series have the same excitation polarisation. The combination of two colours and two polarisations allows us to monitor the distances between single molecules using Fluorescence Resonance Energy Transfer (FRET), as well as the orientation of the molecules. The microscope uses TIRF illumination, which restricts fluorescence excitation to a layer a few hundred nm thick, reducing the level of out of focus background fluorescence and allowing single molecules to be detected.

The microscope can be used to image single fluorescent molecules in live cells, allowing the monitoring of molecular interactions in real time (Fig. 3).

3 colour single molecule TIRF microscope. This microscope is similar to the instrument described above, but allows the imaging of three different fluorophores simultaneously. These multicolour microscopes are required for the investigation of macromolecular interactions in complex networks, for example signalling pathways. As a future development of Octopus, a five colour microscope will be commissioned (funded through a BBSRC Long and Large grant awarded jointly to STFC and King's College London).

http://www.scitech.ac.uk/PMC/PRel/STFC/RALKings.aspx

Confocal microscopy. Octopus offers two confocal microscopy systems. One offers multiphoton FLIM imaging capability to allow the imaging of molecules deep inside living cells. This system has recently been used in a collaborative programme involving STFC and a number of universities, investigating the effects of photodynamic cancer therapy on tumour cells.

http://www.scitech.ac.uk/PMC/PRel/STFC/Sticky.aspx

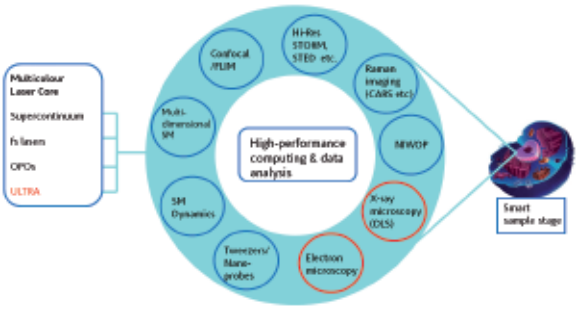

Figure 4. The Octopus concept.

Octopus also has a multicolour confocal microscope that can be used to characterise molecular interactions in live cells. The microscope has recently been upgraded with the addition of a Fianium Supercontinuum laser source, which offers almost limitless flexibility in the choice of fluorescent probes that can be used. Fluorescence correlation imaging is currently being commissioned on this microscope.

Image analysis. The analysis of data from single molecule measurements, particularly in live cells, is challenging because the images often have high backgrounds, signals are weak, and the molecules are mobile. The Functional Biosystems Imaging Group has an active programme of development of advanced image analysis methods, using Bayesian techniques. This programme has been funded through a series of research council grants and the software is made available for use by the user community as it is developed.

The future

The full Octopus cluster will come into operation following the move to RCaH. The new location will allow the complete integration of all the microscopy stations and laser sources, offering a fully flexible, large scale imaging facility to the user community. The vision for Octopus is illustrated in Fig. 4.

Additional microscopy stations will be added as techniques and technology develop, and as the requirements of the community evolve. The location of the Molecular Structural Dynamics arm of the New LSF within RCaH will also allow the formation of an imaging "supercluster", in which large scale pulsed laser systems will be used to image specific molecules in cells without the requirement for labelling.

Octopus will also interact with other facilities on the campus, particularly Diamond Light Source and the proposed Imaging Solutions Centre. Proposed programmes include, for example, the combination of fluorescence, electron, and X-ray microscopy on the same sample, supported by information from macromolecular crystallography and molecular dynamics simulations. This powerful combination, unique to the Harwell campus, will allow us to obtain a complete picture of how the structure of biological macromolecules enables them to fulfil their specific roles in the complex systems that are responsible for the functioning of cells and organisms in health and disease.

References

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