Super-Resolution Developments in the Octopus Facility

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Introduction
The Octopus facility, based in the Research Complex at Harwell, consists of a cluster of advanced laser imaging stations. The facility is accessed by the UK academic community, and also by European academics through the EU-funded “Laserlab-Europe” programme. More recently the facility has been used by a number of UK companies, assisted by STFC’s Collaborative Research and Development scheme. The facility is used mainly for life sciences research, in topics such as plant biology, cell signalling, virus infection, enzyme catalysis, and biomaterials. The first phase of Octopus stations, commissioned on installation in the Research Complex in 2010, included multiphoton confocal imaging with fluorescence lifetime imaging, total internal reflection (TIRF) microscopy, laser tweezers, and a set of microscopes for single molecule tracking and analysis. This article describes Octopus’s second phase of microscopy stations, which have been funded by STFC’s partner Research Councils and commissioned in the last year.

The new stations have introduced “super-resolution” imaging capability to Octopus. This is a collective term for a range of imaging techniques that have been developed to beat the optical diffraction limit, which restricts the resolution of conventional optical microscopes to around half the wavelength of light (about 200 nm). Super-resolution provides up to 10 times better resolution, allowing for much better observation of the molecular machinery of cells.

Stimulated Emission Depletion Microscopy (STED)
Scanning microscopy techniques raster a focused beam of light over the sample and fluorescence is collected during this process to build up an image. The resolution of this type of microscopy is determined by the size of the spot to which the beam can be focused. STED effectively reduces the size of the spot by overlaying the fluorescence excitation beam with a doughnut-shaped beam from a second laser, that “knocks out” fluorescence from the area surrounding the centre spot1 (Figure 1).

Figure 1. Fluorescence excitation beam (left), de-excitation beam (centre), and area of fluorescence emission (right) (Image courtesy of Marcel Lauterbach).

The STED technique allows us to achieve a resolution of 50 nm. Figure 2 shows a comparative image of fluorescently labeled tubulin in cells. The sharper image from STED is a result of the higher resolution. The STED microscopy station was funded by BBSRC.

Figure 2. Fluorescently labeled tubulin imaged in cells using conventional confocal microscopy (left) and STED (right).

Stochastic Optical Reconstruction Microscopy and Photoactivated Localization Microscopy (STORM/PALM)
These closely related techniques achieve super-resolution using the principle of single molecule localization. In an optical microscope, a single fluorescence emitter is visualized as a spread-out spot. However, if the signal-to-noise is good enough, it is possible to calculate the location of the emitting molecule (at the centre of the spot) to within around 20 nm. By using special fluorescent probes that can be activated by light, it is possible to repeatedly image a sample, switching on only a certain number of the fluorescent molecules each time. In this way, the location of the molecules can be determined and a super-resolution image assembled with 20 nm resolution2,3. Figure 3 shows tubulin imaged using the technique.

Two microscopes with STORM/PALM capability were funded by MRC. In addition, a development programme was funded to develop the technique with the ultimate goal of achieving < 20 nm resolution in 3D and in real time, using multiplane imaging and adaptive optics (collaboration with Heriot Watt University and STFC’s UK Astronomy Technology Centre).

Figure 3. STORM image of Fluorescently-labelled tubulin.
Structured Illumination Microscopy (SIM)

MRC have also funded a SIM station on Octopus. With this technique, the sample is illuminated with a pattern, usually the image of a grating. Doing this allows the use of computational methods to remove out-of-focus blur. Frequency mixing of the illumination pattern with the sample caused by the moiré effect results in a downmodulation of fine sample detail into the frequency-support region of the detection optical transfer function. High-resolution SIM achieves typically a twofold lateral resolution enhancement over conventional methods.

Figure 4. Chromosomes imaged by conventional widefield microscopy (left) and SIM (right) (Image courtesy of Christophe Lynch).

Alternating Laser Excitation FRET

The final development this year on Octopus is not an imaging technique, but uses the ability to detect fluorescence from single molecules to obtain information about the structure of molecules and molecular complexes. Förster Resonance Energy Transfer (FRET) is a well-established technique that uses the non-radiative transfer of energy between two fluorescent molecules to obtain distance information in the sub-10 nm range. FRET measurements can be obtained from single molecules, either attached to glass cover slips or in solution, the latter being achieved through using a confocal set-up and a very low sample concentration, the result being that only a single molecule or molecular complex is likely to be in the illuminated volume at any time. Alternating Laser Excitation (ALEX) is a development that uses two alternating laser sources to excite fluorescence from each member of a FRET pair. This allows not only accurate distance measurements but also measurement of stoichiometry. The technique is being used by a number of Octopus users to characterize biomolecular systems such as macromolecular assemblies and chaperone proteins. Figure 5 shows the output from a typical ALEX-FRET experiment.

Figure 5. ALEX experiment measuring the distance between two labeled residues on a protein. FRET efficiency is plotted on the axis labeled “Epr” and possibly indicates multiple states, one with a FRET efficiency around 0.3, with evidence for higher FRET efficiency states extending to around 0.8. This may correspond to variations in the conformation of the protein.

Conclusions

The availability of these new techniques makes Octopus one of the most advanced optical microscopy facilities in the world, and add to the wide range of imaging techniques available on the Harwell Campus. Support for users includes sample preparation and data analysis advice and assistance as well as assistance with data collection. Potential users are invited to discuss applications with the Octopus support team.

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References