A brief history of the octopus imaging facility to celebrate its 10th anniversary

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Summary
Octopus (Optics Clustered to OutPut Unique Solutions) celebrated in June 2020 its 10th birthday. Based at Harwell, near Oxford, Octopus is an open access, peer reviewed, national imaging facility that offers successful U.K. applicants support to single molecule imaging, confocal microscopy, several flavours of superresolution imaging, light sheet microscopy, optical trapping and cryoscopy electron microscopy. Managed by a multidisciplinary team, Octopus has so far assisted >100 groups of U.K. and international researchers. Cross-fertilisation across fields proved to be a strong propeller of success underpinned by combining access to top-end instrumentation with a strong programme of imaging hardware and software developments. How Octopus was born, and highlights of the multidisciplinary output produced during its 10-year journey are reviewed below, with the aim of celebrating a myriad of collaborations with the U.K. scientific community, and reflecting on their scientific and societal impact.

Once upon a time...
In the 1990s, two of the U.K.’s large-scale facilities, the Synchrotron Radiation Source (SRS) at the Daresbury Laboratory and the Central Laser Facility (CLF) at the Rutherford Appleton Laboratory (RAL), began to incorporate fluorescence imaging facilities into their much larger user programmes. The facilities at the SRS focused on total internal-reflection fluorescence (TIRF) microscopy, single molecule fluorescence resonance energy transfer (FRET) and single molecule polarisation imaging, leading to the development of combined single molecule FRET and polarisation microscopy. In turn, the facilities at RAL incorporated confocal laser scanning microscopy (CLSM), fluorescence lifetime imaging (FLIM), and optical trapping using laser tweezers and tweezer nanoprobes, the latter integrated into both fluorescence and Raman microscopes.

In 2007, the SRS replacement, the Diamond Light Source, began user operations at Harwell. The SRS closed in August 2008. The Science and Technology Facilities Council (STFC), which operated both the SRS at Daresbury and the CLF at RAL decided to relocate the SRS single molecule imaging facilities to RAL to be managed jointly with the existing CLF facilities. In early 2010, both were deployed into the newly built Research Complex at Harwell (RChA). In June 2010, they began to operate jointly under the Octopus name, which was inspired by the appearance of the TIRF facilities at Daresbury, in which big lasers were shared modularly between surrounding single molecule microscopes via the use of optical fibres, thus resembling an Octopus. The nascent Octopus preserved at the RChA the legacy accumulated over previous years and combined this with an enticing portfolio of new in house developments. One branch focused on multicolour single particle localisation and tracking methodology. A second branch focused on advancing CLSM techniques by combining phosphorescence lifetimes imaging microscopy (PLIM) with FLIM, and developing two-photon FRET techniques in combination with optical trapping methodology. These technique combinations have proved popular from the onset, as evidenced by the ~3 fold oversubscription throughout a decade of operations.

Since 2010, Octopus growth was backed by £6.5M from research councils responsive mode grants. Via this funding, Octopus acquired several commercial superresolution microscopes, including two-dimension (2D) and three-dimension (3D) stochastic reconstruction optical microscopy (STORM), gated 2D and 3D stimulated emission depletion (STED). 3D structured illumination microscopy (SIM), confocal Airyscan, and laser light sheet microscopy, which were customised according to user needs.

The most recent endeavour is in cryofluorescence microscopy. By the incorporation of solid immersion lenses, Octopus scientists doubled the numerical aperture possible at...
liquid nitrogen temperatures.\textsuperscript{21} The recent commissioning of a cryo focused ion beam scanning electron microscope has spearheaded initial efforts in cryocorrelative light and electron microscopy methods. The long-term aim is to exploit the strategic advantage brought about by the colocalisation of Octopus on the Harwell Campus with the electron bioimaging centre (eBIC) and the cryoimaging X-ray imaging facilities at the Diamond light source.

Within the bosom of the Octopus

Although many R&D endeavours in Octopus have had their foundations in biology, the science deployed by the facility has cut across fields and disciplines. Indeed, the diversity of the questions addressed has required a multidisciplinary working ethos, which involved mastering preparation and analysis methods for many types of biological and nonbiological samples. These included cultured mammalian cells, primary plant cells, yeast, Gram-positive and Gram-negative bacteria, parasites, 3D spheroids, human tissue sections, 3D printed biotissues, small embryos, DNA, protein scaffolds, 3D matrices, aerosols, nanocrystals, polymer blends, inorganic nanocomposites, nanoceramics and aerosols. Labelling methods were developed in house to, for example, minimise glass contamination in TIRF imaging,\textsuperscript{22} leading to the discovery of a rule that links hydrophobicity, nonspecific labelling and the structure of the dye.\textsuperscript{23} Application-specific probes were also synthesised. For example, fluorescence biopolymers for multiphoton FLIM and superresolution imaging,\textsuperscript{24} and polymer gold nanoparticle composites for surface-enhanced Raman spectroscopy and scattering.\textsuperscript{25} The variety of problems has often required exploiting combinations of imaging methods with exhaustive data analysis efforts, including in some instances, developing new analysis routines when commercial programmes were not available.\textsuperscript{26,27,12} A cartoon commissioned by the CLF to celebrate Octopus’ 10th Anniversary is shown in Figure 1.

Multidisciplinary efforts across disciplines paid off by bringing new opportunities for interaction and collaboration. In any given week, one could find up to four research groups from different labs accessing different instrumentation and methods. As a result, it would not be rare for environmental chemists using optical trapping to rub shoulders with embryologists using light sheet microscopy, for physicists designing novel nanomaterials to discuss superresolution imaging with bacteriologists, or for cancer scientists to discuss the merits and drawbacks of single particle tracking (SPT) with plant biologists. Indeed, daily cross-fertilisation between fields, disciplines and techniques was an important mechanism by which the Octopus user community became aware of relevant advances made in other disciplines at the earliest opportunity.

What follows is an account of some of these accomplishments. For reason of space, this review could not be exhaustive. Rather, it aims at giving a flavour by selecting examples across different fields, tackled with a range of imaging techniques, and from academics based in different U.K. locations. Examples have been grouped according to the field of enquiry. In the first three sections I review some of the impact that Octopus achieved in plant biology using the combination of TIRF, SPT, CLSM, FLIM and FRET. I then touch in the next two sections upon some achievements in mammalian cell signalling using SPT, single molecule localisation methods, CLSM, FRET and molecular dynamics (MD) simulations. This is followed by two sections of highlights in medical research, using superresolution and two-photon CLSM, respectively. I finish this review with a section on how some of the life sciences-inspired developments in Octopus came full circle to cross-fertilise physics and chemistry, thus underpinning the impact Octopus has had in determining the nanoscale organisation of novel materials.

To TIRF or not to TIRF... in Arabidopsis cells

Unlike animal cells, plant cells are surrounded by a cell wall that is made up of various sugars such as cellulose.\textsuperscript{28} The cell wall is a tough, yet flexible structure that provides structure, protection, and permeability to the cells. The cell wall is also used to maintain cellular pressure and prevent the cells from overexpansion. Prior to the birth of Octopus, the plant biology community had believed that TIRF illumination,\textsuperscript{3} which removes autofluorescence background from deeper regions of the cells, could not be exploited in plant cells because the cell wall would restrict the penetration of the evanescent field and lead to scattering of illumination. An early example of cross-fertilisation between fields, and benefiting from a wealth of in house experience in TIRF microscopy of mammalian cells, in the first year of Octopus operations, Zoe Wilson’s group (Nottingham University) reported that TIRF imaging was possible in root cells of \textit{Arabidopsis thaliana}, one of the model organisms used for research on numerous aspects of plant biology. Indeed, TIRF was possible not only at the cell plasma membrane but also in organelles, for example the nucleus, due to the presence of the central vacuole.\textsuperscript{29} TIRF images of the \textit{Arabidopsis} root cells, including unlabelled controls (Fig. 2A), labelled microtubules (Fig. 2B), plasma membrane, (Figs. 2C, D) and vacuoles (Fig. 2E), showed the highest signal-to-background ratio in plant cells to date. The latter is illustrated in Figure 2, where TIRF images are compared with images obtained using conventional epifluorescence. This work also compared TIRF illumination with variable-angle epifluorescence microscopy (VAEM), a technique that uses a narrow band of illumination (not an evanescent field) which penetrates into the sample and passes through it almost parallel to the coverslip. At the time, VAEM was also relatively uncommon in plant biology, having been used to image in vivo dynamics of secretory vesicles in pollen tubes,\textsuperscript{30} and to study the dynamics and function of dynamin-related proteins, which are required for cytokinesis and cell expansion.\textsuperscript{31} Results showed that TIRF also provided a higher signal-to-
The ability to do TIRF and single molecule microscopy was a fundamental change in plant biology because it opened up the plant field to SPT investigations of molecular interactions in vivo.

Staring at the wall of Arabidopsis root cells ... whilst tracking single molecules

Indeed, hard on the heels of the above seminal TIRF work, John Runions and coworkers (Oxford Brooks) exploited SPT methods in Octopus to investigate in Arabidopsis root cells the anomalous (i.e. non-Brownian) diffusion of membrane proteins. In both animal and plant cells, plasma membrane proteins continually diffuse along the cell-limiting lipid bilayer, becoming immobilised at specific locations to orchestrate protein–protein interactions critical to the transduction of incoming signals in response to environmental cues. In animal cells, anomalous diffusion and protein immobilisation results from a combination of structuring factors that were known to involve an array of membrane-spanning proteins that maintain plasma membrane microstructure in association with the cytoskeleton. The Runions group showed that in plant cells the plasma membrane immobilisation mechanisms were different to those in mammalian cells, with protein diffusion being in the former constrained by the cell wall. Follow-on research determined that the cell wall takes on the same role played in mammalian cells by membrane-spanning proteins and the cytoskeleton, which is to regulate the dynamics and size of plasma-membrane nanodomains. This was another example of the advantages of cross-fertilisation between plant cell biology and single particle imaging of mammalian cells, and an important moment in plant biology, in which our understanding of the cell wall transformed from being an outer protective layer of cellulose to having a functional role in the receipt and transduction of plant cell signals.

‘A life of loving plants’ under the confocal microscope

The group of the late Chris Hawes (Oxford Brookes), a long-term Octopus collaborator, made pioneering discoveries on the interactions between the endoplasmic reticulum (ER) and the Golgi in Arabidopsis cells. The method of choice was a combination of optical trapping with CLSM, FLIM and FRET, and recently TIRF. Collaborative efforts revealed many details of the intertwined function of these organelles, and produced some of the most impactful images and videos made in Octopus.

The ER is a large, continuous membrane-bound organelle present in all eukaryotic cells. The multiple functions of the ER, including protein synthesis, folding, modification and transport, are facilitated by its compartmentalisation into structurally distinct domains, like high-curvature tubules, peripheral cisternae, where ribosomes concentrate, and contact sites with other organelles, including the Golgi. A number of membrane-shaping proteins regulate the membrane curvature of these ER domains. Reticulons (RTNs) are a class of proteins that help shape the ER membrane into tubules. Previously, it was thought that RTNs could bend the ER membrane via a combination of wedging and scaffolding arising from the ability of RTNs to form low-mobility homo-oligomeric arcs. However, a collaborator of Chris Hawes, Lorenzo Frigerio (Warwick University), and colleagues identified in plant cells a region in the C terminus of RTNs containing a putative amphipathic helix. These lipid-binding helices insert themselves in the membrane, and can therefore participate in membrane remodelling by sensing and/or inducing curvature. Using the combination of CLSM, FLIM and FRET, Frigerio and coworkers studied the plant RTN isoform RTN13, which had previously been shown to locate to ER tubules and the edges of ER cisternae. The results showed that deletion or disruption of the hydrophobic face of the predicted helix abolishes the ability of RTN13 to induce constrictions of ER tubules in vivo without affecting RTN oligomerisation. These findings indicate that proteins of the RTN family use an amphipathic helix to affect membrane curvature, a mechanism shared by several other membrane-shaping protein families.

The most recent breakthrough by Chris Hawes’ colleagues was the discovery of the puzzling location of the Arabidopsis ER-α-mannosidase I (MNS3), a crucial enzyme responsible for the last N-glycan processing step evolutionary conserved across yeast, mammals and plants. N-glycosylation, whose primary function is to enhance the solubility of
**Fig. 2.** Arabidopsis roots imaged using epifluorescence and TIRF. (A) Wild-type control; (B) MAP4–GFP (microtubule marker); (C) LIT6b–GFP (plasma membrane marker); (D) N84725 (PIP2a; plasma membrane marker); (E) N84727 (Delta TIP; vacuolar membrane marker). Scale bars in columns 1 and 2 = 10 µm. Scale bars in details = 5 µm. Reproduced with permission from Ref.29.

| Epifluorescence | TIRF | (High magnification detail) |
|-----------------|------|-----------------------------|
| **(A)** Wild type |      |                             |
| **(B)** MAP4-Microtubules |      |                             |
| **(C)** LIT6b-Plasma membrane |      |                             |
| **(D)** PIP2a-Plasma membrane |      |                             |
| **(E)** Delta TIP-Tonoplast |      |                             |

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protein folding intermediates, begins at the ER by transferring the preassembled oligosaccharide precursor Glc3Man9GlcNAc2 en bloc onto selected asparagine-led sequences of the nascent polypeptide chain (Fig. 3A). N-glycans are then processed in a highly concerted manner by a cohort of ER- and Golgi-resident glycosyl-transferases and glycosidases.\[^{43}\] The resulting Man9GlcNAc2 oligosaccharide is processed by MNS3, which removes a single terminal mannose residue from Man9GlcNAc2. A putative ER localisation of MNS3 in plant cells was inferred because the yeast ortholog Mns1p resides in the ER.\[^{44}\] Schoberer and colleagues, however, showed that in plants, a fluorescent protein fusion of MNS3 resides in the Golgi (Fig. 3B). Confocal images, like those in Figure 3(C), showed MNS3 is retained by an amino acid signal motif (LPYS)\[^{42}\]. The physiological consequence is the spatial separation of the MNS3-mediated N-glycan processing steps from ER-located trimming steps that generate the glycan signal required for flagging terminally misfolded proteins for ER-associated degradation (ERAD). This may serve as a checkpoint for the separation of ERAD substrates and ER residents from secretory cargo that receive further processing in the Golgi, of critical importance for cell homeostasis.

**Using SPT to capture pros and cons of cancer drugs against the mammalian EGFR family**

A popular biological target to investigate cell signalling in Octopus was the epidermal growth factor receptor (EGFR). This receptor is the tyrosine kinase archetype and plays a fundamental role in the regulation of cellular growth.\[^{45}\] In all vertebrates, EGFR signals in concert with three other receptor homologues, which in humans are known as human epidermal growth factor (HER)1-4.\[^{46}\] Homo and hetero HER dimer and oligomer formation are at the heart of the signalling networks that promote organ-specific cellular growth, survival, migration, and differentiation. Dysregulation of these interactions is responsible for cellular transformation in different organ cancers.\[^{47}\] One of the challenges is that HER-targeted
anticancer therapies can also promote homo and hetero associations in a similar manner as the natural HER ligands, and this might be associated with the development of drug resistance. Thus, understanding how interactions among HER family members with therapeutic drugs alter growth factor signalling biology is fundamental to suggest avenues for overcoming resistance to anticancer therapy.

A programme of developments was undertaken in Octopus to investigate homo and hetero interactions of members of the HER family via two and three-colour SPT, and longer range clustering via STORM. Unlike previous two-colour SPT work, which used quantum dots to label the EGFR family, Octopus scientists focused on tracking receptor particle colocalisation at low protein expression, which is better achieved using organic dyes. It was known for some time that the formation of HER4-HER4 homo-complexes could slow the growth of some breast cells, producing a more differentiated epithelial phenotype. Thus, overexpression of HER4 in a breast tumour can signify a positive prognosis. HER4 is alone in the HER family in having alternative spliced variants that encode two cytoplasmic isoforms (Cyt1 and Cyt2). The ectodomain of the HER4-Cyt2 is cleaved via the activity of tumour necrosis factor-alpha converting enzyme (TACE/ADAM17), leaving behind the intracellular domain tethered to the plasma membrane. Using SPT, Tony Ng and coworkers (King’s College London) showed that the cleaved HER4 Cyt2 variant, by protecting HER1 from ligand-induced degradation, leads instead to enhanced cell motility and migration, with consequences for the onset of metastasis. These results highlighted the different prognosis value of the two HER4 isoforms for the analysis of breast tumours in the clinic.

In contrast, the overexpression of HER2 strongly correlates with the prognosis of breast cancer. HER2-HER 3 hetero complexes produce potent proliferative and antiapoptosis signals widely implicated in the resistance to the common HER2-mediated therapy lapatinib, an ATP-competitive inhibitor of HER2, hailed as a ‘tumour-busting wonder drug’ for the treatment of breast cancer. However, a significant proportion of patients develop progressive disease due to acquired resistance. Using the combination of SPT, STORM and FRET-FLIM, Peter Parker’s group (Francis Crick Institute) showed that lapatinib cooperates with the natural ligand of HER3, neuregulin, to drive HER2-HER3 hetero-oligomer formation via a previously unknown interface. This afforded a proliferative outcome and explained the development of drug resistance. These mechanistic insights into the liabilities involved in targeting kinases with ATP-competitive inhibitors and highlighted the complex role of protein conformation in acquired resistance.

Using single molecule localisation to get our heads around structure–function connections

Protein structure determines function. Arising through natural selection, structure–function relationships are normally predicted by cell-free structural determination methods, but hypotheses often have to be tested in cells. This typically requires imaging resolutions comparable to the size of the molecules of interest, so that the formation of multiprotein complexes, crucial to deploy rapid but controlled responses to incoming cues, can be interrogated. A traditional method of choice is FRET, a spectroscopic ruler that measures inter- and intramolecular separations in the 1–10 nm range. FRET has extensively been used, including by Octopus scientists, to investigate structure–function relationships in the EGFR field. Some FRET-derived pioneering findings include the use confocal FLIM-FRET to discover EGFR lateral signal propagation. In turn, single molecule FRET was used to reveal the timing of the association between EGFR dimerisation and phosphorylation, whilst the combination of FRET and image correlation microscopy was exploited to reveal the existence of EGFR oligomers. However, FRET can present significant limitations to study receptor interactions. Because the lateral size of the EGFR is ≥ 5 nm, which is not uncommon in membrane proteins, often even first neighbour interactions fall outside the 1–10 nm range of FRET. Dimers and oligomers are in turn too small for their architecture to be resolved by STORM (typically ~20 nm resolution). A programme of developments in Octopus, involving a collaboration between astronomers and biologists, focused on closing the resolution gap between superresolution imaging and FRET.

One possibility was to exploit the ability of single molecule imaging to localise individual molecules by their fluorescence emission to 1–2 nm precision. The rationale was that this could be used to identify the positions of fluorescence-labelled proteins in oligomers to reveal stoichiometric and architectural information. This could be achieved, for example, by localising individual fluorophores in oligomer image spots from the change in position of the spots upon fluorophore step-photobleaching events. Coined fluorophore localisation with photobleaching (FLImP), the method achieved <5 nm lateral separation resolution and can measure intermolecular separations in oligomers as large as 60 nm. To attain a 3D perspective of the architecture of the oligomer complexes at the membrane, the FLImP method was combined with CLSM and FLIM-FRET, the latter used to measure the vertical distance from the fluorophores to the membrane.

Exploiting the <5 nm resolution of FLImP to constrain long duration MD simulations from the D.E. Shaw Research group, Octopus scientists experimentally validated the atomic resolution structural model of EGFR oligomers in cells when bound to its cognate ligand epidermal growth factor. The results showed that oligomerisation was essential to organise receptors in ways optimal for trans-activation, findings, which questioned the dimer-centric paradigm until then deeply rooted in the field.

FLImP also removed another obstacle by describing, again in collaboration with the D.E. Shaw Research group, the in situ structure of unliganded inactive dimers and oligomers.
The inherent molecular compounds against multidrug resistant complexes were exploited, however, less effective against Gram-positive bacteria like *Staphylococcus aureus* SH1000 and other isolates. This included MRSA, which is responsible for many antibiotic-resistant hospital-acquired infections. STED experiments revealed that lead RuII compounds do disrupt the membrane structure of Gram-positive bacteria before internalisation, which ultimately results in a small amount of DNA damage. Further analysis showed that increased expression of the multiple peptide resistance factor (*mprF*) gene was behind strain-specific resistance to the RuII compounds. The *mprF* gene, which also plays a crucial role in antibiotic resistance, encodes a bifunctional membrane protein that catalyses the synthesis and translocation (flipping) of the positively charged phospholipid lysyl-phosphatidylglycerol. Overexpression of *mprF* leads to accumulation on the outer leaflet of the cytoplasmic membrane of positive charge, thereby providing enough protection against cationic antimicrobials, like the RuII complexes. Consistent with this model, it was found that an *mprF* deficient strain was particularly susceptible to treatment with the lead complex. These results highlight the challenges associated with the development of new antimicrobials.

Octopus also made inroads in the investigation of protozoan parasites. One example is *Toxoplasma gondii*, which causes...
Fig. 5. (A) Localization in *E. coli* EC958 cells of the $4^{4+}$ dinuclear Ru$^{II}$ compound shown in (B), visualised through stimulated emission depletion (STED) nanoscopy. (B) Structure of the most active dinuclear Ru$^{II}$ complex against Gram-negative bacteria. Figure reprinted with permission from Ref$^{70}$. Copyright 2019 American Chemical Society.

toxoplasmosis, an infectious disease of particular importance in pregnant women, where it can lead to severe symptoms or even death of the foetus.$^{73}$ *Toxoplasma gondii* is an obligate intracellular one-celled eukaryote that replicates via an unusual process, described as internal budding, by which multiple daughter parasites replicate sequentially within a single mother cell (Fig. 6A). The expectation in the field was that new cohorts of sub-cellular organelles would be created *de novo* in each developing daughter parasite. However, using 3D SIM, Javier Periz and colleagues from Glasgow University showed that organelles, like the secretory (micronemes), are instead recycled from the mother to the daughter parasites via a highly dynamic F-actin network.$^{74}$ This actin network supports long-range multidirectional vesicular transport between mother and daughters, and regulates density and localisation of micronemal vesicles (Figs. 6A–F). The actin connectivity ensures that the parasite uses its resources highly economically by recycling maternal material. These insights revealed a general mechanism of organelle replication in apicomplexan parasites, like *Toxoplasma gondii*, suggesting a new approach for therapeutics.

Invisible light to crush the heart of melanoma tumours via two-photon CLSM

The substantial in house expertise in combining radiation damage and two-photon CLSM$^{75}$ together with cross-fertilisation between chemists and biomedical scientists treatments facilitated success in, for example, investigations.
of photodynamic therapy (PDT), a still emerging treatment that involves the photooxidation of medicinal compounds accumulating in cells and tissue.\textsuperscript{76} By focussing light into the developing tumour, abnormal cells can be selectively destroyed among surrounding healthy tissue. However, due to rapid growth, many tumours possess hypoxic regions, particularly at and around their core. Thus, incomplete treatment of tumours by PDT leading to relapse, often involves the hypoxic regions, far enough into the heart of the tumour to make them unreachable. One possibility is to use compounds that can be excited with near-infrared (NIR) light, which can penetrate much deeper into tissue.\textsuperscript{77} The dinuclear Ru\textsuperscript{II} complexes discussed above can be photoxidised and are readily taken up by melanoma-derived cells, where they localise in mitochondria and the nuclei. The group of John Haycock (Sheffield University) showed that these Ru\textsuperscript{II} complexes can be activated by NIR light using two-photon excitation.\textsuperscript{78} PDT offers particular potential for the treatment of skin cancer, such as melanoma, which is by its nature more accessible to photonic methods.\textsuperscript{79} By loading the Ru\textsuperscript{II} compounds into 3D melanoma spheroids, which contain healthy, hypoxic and necrotic layers, the characteristic emission of these complexes was used as a probe to investigate their uptake. Results using CLSM revealed that by using two-photon absorption at a power of 60 mW, which was progressively focused in 10 µm steps throughout the entire z-axis of individual spheroids, rapid photoinduced cell death was observed throughout the 280 µm depth of the spheroid, including the hypoxic region. These results revealed a highly promising lead for focused PDT regimes.

Going full circle: is there such thing as a free lunch in advanced materials research?

Cross-fertilisation between disciplines is more commonly exemplified by the application of physics techniques to the life sciences. In Octopus, cross-fertilisation went full circle by deploying biology-inspired analysis methods to answer fundamental physics and chemistry questions. The relative simplicity and speed with which Octopus made pioneering contributions to these fields was astonishing, and it stands as an exemplar of the power of cross-fertilisation to facilitate ground-breaking work.

One example is the application of optical trapping methods to control 3D block copolymer self-assembly. The latter has enabled the creation of a range of solution-phase nanostructures with applications from optoelectronics and biomedicine to catalysis, and can produce unique nanostructures under soft confinement.\textsuperscript{80} In collaboration with the group of Ian
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optical tweezers were used to trap, manipulate and pattern individual cylindrical micelles and larger hybrid micellar materials. Through the combination of TIRF imaging and optical trapping, the 3D motion of individual cylindrical block copolymer micelles in solution was precisely controlled (Fig. 7). Automation of this process enabled the creation of large ordered customisable arrays of complex hybrid block copolymer structures containing hundreds of assemblies, the process completed in minutes rather than hours, thus allowing quick fabrication.

A second example is the design of 2D materials. Typified by graphene, the outstanding physical and chemical properties of 2D materials are in principle easier to control in assemblies engineered from soft matter, like block copolymers. However, the formation of 2D platelet micelles is generally uncommon relative to 3D block copolymers morphologies. In another successful collaboration with the group of Ian Manners, using CLSM and SIM, Qiu et al. showed these complex nanoobjects can be subject to spatially selective processing that allows their disassembly to form perforated platelets, such as well-defined hollow rectangular rings. The sought after 2D geometries were achieved by means of seeded-growth methods that involve the sequential addition of different blends of crystalline-coil block copolymers and the corresponding crystalline homopolymer to cylindrical micelle seeds. These results provided a tuneable platform for further functionalisation and potential for a broad variety of applications.

A third example is single crystal nanoparticles, an attractive class of nanocomposite materials where judicious combinations of the host and additive can result in new properties including colour, magnetism and conductivity. Fiona Meldrum and colleagues (Leeds University) demonstrated the utility of 3D STORM to characterise the internal structures of nanocomposites, specifically calcite single-crystals doped with fluorescence quantum dots, which are useful for STORM because of their blinking properties. The STORM images showed that the types of dislocations formed at the crystal/substrate interface vary according to the nucleation face, and that dislocation loops have different geometries to classic misfit dislocations. This novel approach to the study of nanocomposites offered a rapid and nondestructive method for visualising the dislocations present within crystals, and provided new insight into the mechanisms by which additives become occluded.

Last, but not least, Octopus made important contributions in atmospheric and environmental chemistry, for example, by optically trapping pharmaceutical aerosols, which were then analysed using combinations of dispersive Raman microspectroscopy, and FLIM and broadband Mie scattering. These methods allowed, for example, studying the chemistry and optical properties of cloud droplets and pollution, simulating the respiratory environment of pharmaceuticals in the lung, and understanding the viscosity behaviour of airborne droplets.

Concluding remarks

This review celebrates 10 years of science in the Octopus Facility. The Octopus journey has combined a passion for curiosity-driven research with a keen eye for impact, particularly in the fight against human disease. An Octopus journey that has involved pioneering efforts to reveal the structure and architecture on molecular complexes under physiological conditions, painstakingly chipping out information about molecular structure, clustering, interaction rules and transport, organelle organisation, interactions in plant and eukaryotic cells, drug delivery to mammalian cells, investigating tumour models and facilitating the fight against infectious diseases. A journey that has most satisfactorily come full circle to exploit biology-based knowhow on providing crucial nanoscale information on hard-core material and chemical research. This has been the Octopus journey so far. Many happy returns!

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