Imaging & Dynamics for Physical & Life Sciences

DNA double-strand breaks probed with super-resolution imaging nanoscopy

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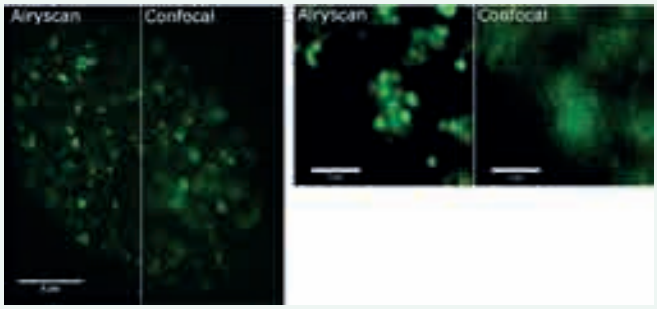
Genomic DNA is continuously damaged by metabolic processes and by external sources, such as ionising radiation. The phosphorylation of histone H2AX on serine residue 139 (described as γ-H2AX) is an excellent indicator or marker of DNA double-strand breaks (DSBs). The yield of γ-H2AX (foci) is shown to have some correlation with the dose of radiation or other DSB-causing agents. There is, however, some discrepancy in the DNA DSB foci yield among imaging and other methods, such as gel electrophoresis.

We have compared the performance of several super-resolution techniques for determining the amount and spatial distribution of γ-H2AX foci formation in the nucleus of cells after x-ray irradiation. The super-resolution imaging methods used include stimulated emission depletion (STED), ground-state depletion microscopy followed by individual molecule return (GSDIM), and structured illumination microscopy (SIM), as well as an improved confocal, Airyscan and HyVolution 2. By using these imaging techniques to achieve resolutions as low as 30 nm, each focus may be further resolved. This increases the number of foci observed per radiation dose compared to standard microscopy, and provides a more reliable quantification of DSBs.



Clear comparison between the foci resolved with Airyscan versus confocal. Fluorescence images of x-ray irradiated Hela cells labelled with γ -H2AX-A488.

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Solid immersion microscopy images cells under cryogenic conditions with 12 nm resolution

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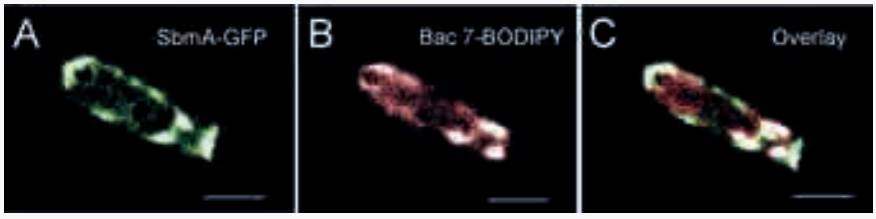
The resolution in conventional cryogenic fluorescence microscopy (~400 nm) is hindered by the use of dry objective lenses. Here we applied a super-hemispherical solid immersion lens (*super*SIL) in conventional cryogenic microscopy to achieve at least 12 nm resolution, in combination with single molecule localisation microscopy. The new technique enables optical imaging at macromolecular level, bridging the resolution gap between optical and electron microscopy.

SuperSIL multi-colour imaging of E.coli cells under cryogenic conditions. (A) Secondary ABC transporter protein SbmA labelled with EGFP; (B) Anti-bacterial peptide Bac7 labelled with BODIPY; (C) The overlay image of the SbmA and Bac 7. Scale bars: 1μm.



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Investigating the processes of cell receptor signal regulation

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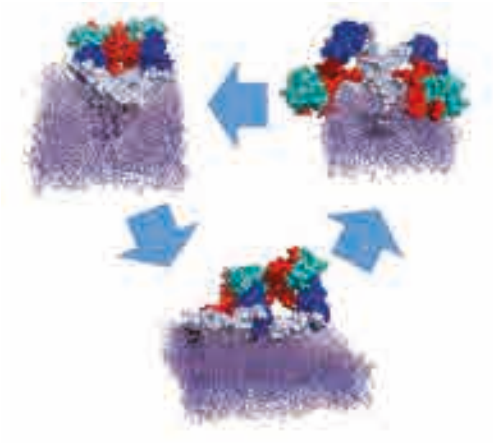
The epidermal growth factor receptor (EGFR) is a protein tasked with transducing external signals across the cell surface, and EGFR activation in the absence of a signal is implicated in breast and lung cancer. Current models of EGFR autoinhibition are based on structural data from receptor fragments and do not explain how mutations achieve signal-independent phosphorylation.

We have used a repertoire of OCTOPUS imaging technologies to study the structure of whole receptors in the cell membrane and this data has been informed with simulations. This has revealed an extracellular head-to-head interaction through which ligand-free receptor polymer chains of various lengths assemble. This head-to-head interaction prevents kinase-mediated dimerization leading to autoinhibition. Mutations of the receptor or certain intracellular treatments split the head-to-head polymers into two different forms of dimers that are either intracellularly active or inactive.

Contrary to the previously proposed models, our results suggest that only dysregulated EGFRs have populations of symmetric and asymmetric kinase dimers that coexist in equilibrium at the plasma membrane under the modulation of the C-terminal domain.



Collage of simulations showing the relations between the inactive 'back-to-back' EGFR dimer (top left) , autoinhibited 'head-to-head' dimer (bottom) and the always active 'stalk-to-stalk' dime.

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Probing Charge-transfer Dynamics of Porphyrin-C_{60} Dyes and Bodipy Polymers for Solid State Tandem Solar Cells

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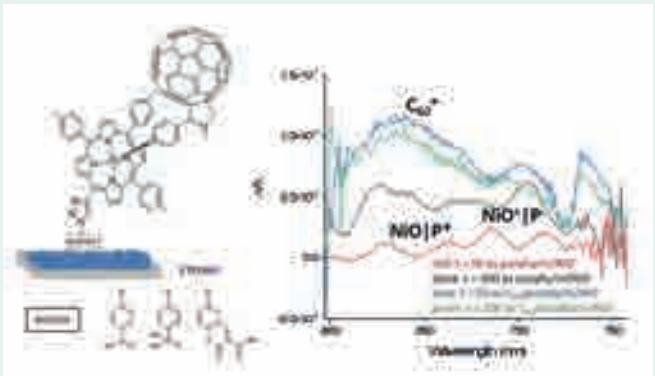
A series of zinc tetraphenyl porphyrin photosensitizers furnished with three different anchoring groups, benzoic acid, phenylphosphonate and coumarin-3-carboxylic acid were prepared using 'click' methodology. Their adsorption behaviour on the electrode surface, kinetics of charge-separation at the dye-electrode and dye-redox mediator interfaces and performance in solar cells is described. The photocurrent of the p-DSCs increased with increasing dye loading and corresponding light harvesting efficiency of the electrodes. Coordinating the zinc to a pyridyl-functionalised fullerene (C_{60}**PPy**) extended the charge-separated state lifetime from ca. 200 ps to 4 ns and a positive improvement in the absorbed photon to current conversion efficiency (APCE) was observed. Finally, we confirmed the viability of electron transfer from the appended C_{60}**PPy** to PCBM, a typical electron transporting layer in organic photovoltaics. This has implications for assembling efficient solid-state tandem solar cells in the future.



Left: Porphyrin-Fullerene conjugates for investigation in this project. The porphyrins on the left are functionalised with different anchoring groups to compare the effect of anchoring on electron transfer from NiO to the Porphyrin. The C_{60} derivative is coordinated through the Zn.
Right: Representative spectra at selected time delays of porphyrin 3 (coumarin anchor) anchored to NiO in the presence and absence of C_{60} to show the relevant intermediates in the cascade.

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Photocatalytic Methanol Degradation on a TiO_2 Surface Monitored by IR-Vis Sum Frequency Generation through a Transparent Electrode Material

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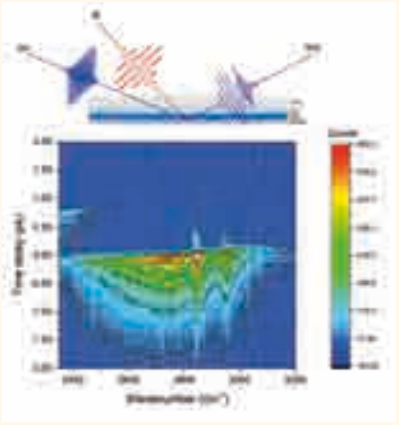
The surface specificity of IR-Vis vibrational sum frequency generation (VSFG) spectroscopy is used to probe a photocatalytically active TiO_2 surface through a transparent conductive oxide (TCO) layer. We demonstrate the feasibility of monitoring a dynamic process in this novel experimental geometry by using methanol as a probe molecule. 355 nm LED illumination is used to carry out in situ photolysis of surface adsorbed methanol species, resulting in a loss in intensity of the corresponding C-H stretching modes in the VSFG spectra.

The presence of a TCO layer also enables electrochemical control of the system, opening the door to exciting applications of *in situ* VSFG spectroscopy to the field of photoelectrochemistry.



SFG spectra of MeOH adsorbed on TiO_2 surface through a multi-layer structure at varying time delays between IR and Vis pulses.

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Mapping Catalysis with Time-resolved Infra-red Spectroscopy

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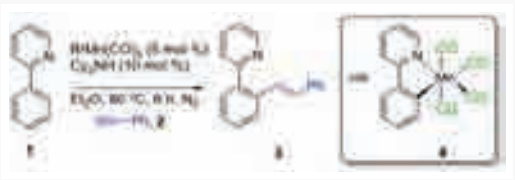
The use of time-resolved infra-red spectroscopy to map the key carbon-carbon bond formation steps underpinning manganese-catalysed C-H functionalisation reactions is described. UV light is used to dissociate a carbonyl ligand from an organometallic manganese catalyst to initiate subsequent interactions with substrates such as alkynes and alkenes. Analysis of the resulting spectra allows for the important interactions between the metal and the substrates to be deconvoluted, as well as the direct observation of the subsequent carbon-carbon bond formation events that underpin the catalytic cycle. The observation of a range of processes was enabled through the temporal flexibility of the Time-resolved multiple probe spectroscopy ($TR^{M}PS$) method.



Scheme 1

Figure 1

Scheme 1. Mn-catalysed C-H functionalisation reaction and structure of key intermediate.
Figure 1: Left TRIR spectra obtained from the photolysis of 4 in neat PhC≡CH and in toluene solution. Right Reaction scheme showing the structures of the complexes formed.
Figure 2: Left TRIR spectra obtained from the photolysis of 4 in neat $^{n}BuCO_2CH{=}CH_2$. Right Reaction scheme showing the structures of the complexes formed.

Figure 2

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