In Single Molecule Localization Microscopy (SMLM), the quality of the super-resolved image highly depends on the capacity to generate single molecule imaging conditions. dSTORM in particular strongly relies on the control of the fluorescent dye photophysics. A method, called ASTER (Adaptable Scanning for Tunable Emission Regions) has been recently proposed to generate an effective uniform excitation by introducing a fast scanning (kHz) of the gaussian LASER beam which creates dynamically a top hat excitation. We will see how this technology can be used to understand and quantify the photoswitching rates for several fluorescent dyes.
We will then present how ASTER can be upgraded by coupling a dynamic zoom, to generate an intelligent excitation pattern that would adapt to the imaged sample. Indeed, high labelling density is a known limitation that can deteriorate the single molecule regime. By previously studying the dye photoswitching response, we can efficiently adapt the scanning pattern of ASTER in these regions. This offers a smart way to redistribute the excitation photons in order to counteract high localizations density. We will show how this technique can be used in time, during an SMLM acquisition, to trigger an optimized and uniform dye photoswitching in the sample.

1347-Pos
Characterizing the structural rearrangement of the bacterial nucleoid by Dps

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Bacteria are subject to a wide variety of environmental stresses which requires a robust stress response system to protect the cell. A key protein in *Escherichia coli* known for its numerous functions in stress response and cell maintenance is the DNA-binding protein from starved cells (Dps). The Dps protein protects DNA via two different mechanisms. First, Dps oxidizes iron to prevent the generation of hydroxyl radicals. Second, in response to stress and throughout the stationary phase of the growth cycle, Dps oligomers bind to DNA and multimerize further to create a Dps-DNA complex that compacts. To access how Dps affects this structural change of DNA to afford protection of the cell's genome, photoactivatable localization microscopy is employed to generate nanometer-scale maps of the nucleoid structure throughout the *E. coli* growth phases. These maps are attained by super-resolving the localizations of single molecules of the nucleoid-associated protein HUα, which non-specifically binds to DNA, tagged with the photoactivatable fluorescent protein PAmCherry. Super-resolution maps of the nucleoid structure are compared to maps of the nucleoid in *E. coli* strains with *dps* deleted. Tagging Dps and other major nucleoid associated proteins with PAmCherry provides the distribution of these proteins across the nucleoid to gain insight on how and if Dps interacts with other nucleoid associated proteins. By observing these structural changes over the growth period of *E. coli*, insight can be gained about how changes in DNA structure can lead to protection via Dps.

1348-Pos
Defect engineering of 2D material for biosensing applications

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2D materials offer huge potential as substrates to build devices for biosensing applications but are plagued by unwanted interactions such as binding/sticking. Controlling such interactions will be critical for the continued exploration of 2D materials in biosensing. In this work, we engineer and tune the surface interactions of hexagonal boron nitride (hBN) to direct the motion and diffusion of DNA. Using ISCAT and fluorescence microscopy techniques, we explore the nanoscopic interactions of DNA with different 2D materials. We show that pristine hBN flakes exhibit the lowest surface interactions and DNA bind preferentially to the edges and regions of high defect density of the hBN flake. We tap into a recently reported Xenon Focused Ion Beam (FIB) technique to engineer edges and defects on hBN flakes. Our technique harnesses a Xenon-FIB to lightly irradiate the desired regions of the hBN flake followed by subsequent etching in water which allows for a much cleaner hBN surface. We are able to enhance DNA binding and affinity at defined locations by inducing defects using FIB. By creating long tracks of defects, we induce diffusion along our created tracks, thereby allowing us to direct motion of the DNA molecules. We envision future devices where such engineered interactions are able to direct biomolecules to sensing regions (such as a nanopore) on 2D material based devices thereby increasing the rate of analyte capture and sensitivity of single molecule sensing devices.

1349-Pos
Nanoscopy on drug-encapsulating nanosystems by phasor-based fluorescence lifetime analysis

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Understanding the supramolecular organization of active compounds (drugs) encapsulated within standard formulations is becoming increasingly important in nanomedicine. In fact, the synthetic identity of a drug-encapsulating nanosystem is supposed to change throughout its journey, from administration to final fate within the target tissue/cell. Here we addressed this issue by exploiting phasor-based fluorescence lifetime imaging (FLIM) of a drug intrinsic fluorescence signal. As case-study, we selected Irinotecan (CPT-11), a chemotherapy medication used to treat colon cancer, small lung cancer and pancreatic cancer, and focused on its liposomal formulation Onivyde®, launched in 2015 as an orphan drug for the treatment of metastatic ductal adenocarcinoma (mPDAC). After measuring the FLIM signature of the drug's individual physical states, we extracted the fractional intensities of each coexisting physical state of the active principle within the nanoparticle. Our analysis was boosted by means of dedicated custom algorithms based on preliminary data processing and multi-dimensional histogram inference to identify regions of interest in the phasor plot. Whereas, in the case of biological samples, we achieved autonomous cell segmentation by means of computer vision algorithms. Onivyde® in commercial vials was found to coexist in multiple physical states (i.e. gelated/precipitated, free and membrane-associated) and undergo extensive leakage in media mimicking complex biological environments. Moreover, we identified multiple mechanism of cellular uptake and were able to investigate subcellular localization and supramolecular organization of irinotecan in a model of pancreatic cells. In conclusion, we believe that Phasor-FLIM represents a useful platform to quantitatively address the synthetic identity of encapsulated fluorescent drugs, both in cuvette and cellular environments. In this context, we envision many potential applications ranging from batch-to-batch analysis, at the production level, to drug development optimization.

1350-Pos
Towards cloud-enabled fully automated end-to-end single molecule imaging

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Single Molecule Super-resolution imaging has revolutionised understanding biological processes on the sub-100 nm scale. Our group developed Fluorophore Localisation Imaging with Photobleaching (FLImP), a super-resolution imaging method that can achieve $<$4 nm resolution. In concert with molecular dynamics simulations, FLImP was used to reveal the *in-situ* atomic resolution structures of ligand-bound EGFR oligomers at the plasma membrane of cells (Needham *et al.*, Nat Comms 2016), and those of ligand-unbound dimers and oligomers on cells (Zanetti-Domingues *et al.*, Nat Comms 2018), shedding new light on the mechanisms of EGFR auto-inhibition, phosphorylation and signalling.
Despite this progress, there are limitations of super-resolution imaging technologies, including: (I) Ability to reliably resolving molecular separations $<$10 nm, (ii) Labour intensive nature of data collection, (iii) Often extremely heuristic (i.e. template led) approaches to molecular structure inference.
To resolve these limitations, we developed a cloud-enabled and fully-automated end-to-end FLImP pipeline. Including region of interest detection, single molecule localisation and tracking, sub-1 nm drift correction, FLImP-suitable object selection and molecular reconstruction. This technique is compatible with an off-the-shelf and self-contained single molecule imaging microscope that does not require an optical table or dedicated laser safety consideration. Use of such instruments, in conjunction with cloud-enabled analysis pipelines has the potential to allow single molecule imaging to be taken out of the specialist microscopy laboratory and into the real-world.
Using this approach, we firstly demonstrate successful and fully automated imaging and accurate reconstruction of ligand-free EGFR dimer separations with sub-10nm resolution in 1D. This technique is then extended to demonstrate the successful reconstruction of 2D molecular structures without requiring substantive structural prior knowledge. This approach is robust to noisy measurements and the inclusion spurious localisations and can be readily applied to other super-resolution techniques and extended to 3D.