Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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if cAMP diffusion is slowed by some other means. To test this hypothesis, we used raster image correlation spectroscopy (RICS) to estimate the diffusion coefficient of gRNA puncta labeled with cyanine-5-cAMP in hA5M cells and found that it is 3.9 ± 0.49 μm²/s, or nearly two orders of magnitude slower than the predicted rate of free diffusion. Disrupting protein kinase A (PKA) interactions with A kinase anchoring proteins (AKAPs) using the anchoring inhibitor H31 nearly doubled the diffusion coefficient of q575-cAMP to 7.9 ± 1.5 μm²/s, suggesting that cAMP movement was being buffered by PKA anchored by one or more AKAPs. Confocal imaging demonstrated that q575-cAMP colocalizes with mitochondria, and Western blot analysis found that D-AKAP2 is the primary mitochondrial AKAP found in hA5M cells. Knockdown of D-AKAP2 expression was achieved using adenovirus-mediated delivery of shRNA, and in knockdown cells EP2R activation was able to stimulate cAMP production in subcellular locations associated with lipid-raft domains, similar to effects previously seen with PDE4 inhibition. These results suggest that buffering of cAMP movement by PKA anchored to the outer membrane of mitochondria contributes to compartmentation of EP2R-mediated responses in hA5M cells.

**Platform: Optical Microscopy and Superresolution Imaging**

**75-Plat**

**Event-driven acquisition for content-enriched microscopy**

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A common goal of fluorescence microscopy is to collect data on specific biological events. Yet, the event-specific content that can be collected from a sample is limited, especially for rare or stochastic processes. This is due in part to photobleaching and phototoxicity, which constrain imaging speed and duration. We developed an event-driven acquisition framework, in which neural-network-based recognition of specific biological events triggers real-time control in an instant structured illumination microscope. Our setup adapts acquisition-on-the-fly by switching between a slow imaging rate while detecting the onset of events and a fast imaging rate during their progression. Thus, we capture mitochondrial and bacterial divisions at imaging rates that match their dynamic timescales while extending overall imaging durations. Because event-driven acquisition allows the microscope to respond specifically to complex biological events, it acquires data enriched in relevant content.

**76-Plat**

**Multicolor super-resolution imaging to study human coronavirus RNA during cellular infection**

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the third human coronavirus within 20 years that gave rise to a life-threatening disease and the first to reach pandemic spread. While the scientific community has studied coronavirus biology using genomics, cryoelectron microscopy, and electron tomography, how coronavirus RNA is spatially organized in the cell at the different stages of the viral replication cycle at nanoscale resolution is largely unknown. To make therapeutic headway against current and future coronaviruses, the biology of coronavirus RNA during infection must be precisely understood. Here, we introduce a multicolor super-resolution (SR) fluorescence imaging framework to examine the spatial interactions between viral RNA and other viral factors during host cell infection. We demonstrate the efficacy of our approach using the HCoV-229E coronavirus in MRC5 lung fibroblasts and specifically label two key oligonucleotide viral players: viral genomic RNA (gRNA) and double-stranded RNA (dsRNA). The 10-nm resolution achieved by our approach uncovers a striking spatial organization of gRNA and dsRNA into three distinct RNA structures: (1) large gRNA clusters, (2) very tiny nanoscale gRNA puncta containing a single copy of the genome, and (3) round intermediate-sized puncta highlighted by the dsRNA label. Furthermore, we use our two-color SR approach to visualize the nanoscale spatial relationships between viral gRNA and the endoplasmic reticulum (ER), dsRNA and ER, gRNA and the spike protein, and gRNA and dsRNA. In particular, we observe two striking observations that provide insight into viral replication and export. First, spike proteins and gRNA rarely assemble into an assembled virion in the MRC5 cytoplasm. Second, in contrast to previous observations, dsRNA and gRNA spatially separate. Our approach provides a comprehensive imaging framework that will enable future investigations of coronavirus fundamental biology and the effects of therapeutics.

**77-Plat**

**In-situ characterization of Alzheimer’s disease tau aggregates via super-resolution imaging**

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Alzheimer’s disease (AD) is among the most common and devastating neurological disorders. One of the pathological hallmarks of AD is the appearance of morphologically heterogeneous insoluble aggregates made up of the microtubule-associated protein tau. The emergence of tau aggregates at distinct brain regions strongly correlates with clinical symptoms and neuropathological features. These observations suggest that tau aggregation has a crucial and toxic role in AD. Tau’s native functions are regulated through the phosphorylation and dephosphorylation of specific residues. Previous studies indicate that insoluble tau aggregates (e.g., neurofibrillary tangles) contain abnormally hyperphosphorylated tau. However, the precise link between the pattern and degree of tau hyperphosphorylation and aggregation remains unclear. Using multicolor DNA-PAINT super-resolution microscopy in human postmortem AD brain tissues, I have mapped specific tau residues that are hyperphosphorylated within tau aggregates, particularly tau oligomers (20-30 nm), which are thought to seed tau aggregation. Preliminary results show that multiple tau residues, including early (phospho-Thr231) and late (phospho-Ser202/Thr205) tau aggregation phosphorylation markers, are enriched within oligomeric tau seeds. In contrast, these phosho-tau marks are differentially enriched within linear and branched tau fibrils. These results suggest that hyperphosphorylation at distinct tau residues may template the aggregation of tau into distinct tau aggregate species. Overall, this work has the potential to facilitate the development of therapies that can accurately target various tau aggregates through the identification of the specific molecular signatures of tau seeds and tau aggregates present in human pathology.

**78-Plat**

**Direct observation and classification of heterogeneous protein aggregation in real-time through super-resolution and aggregational fingerprinting**

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Protein aggregation and misfolding are highly diminishing components of protein function and expression and are known to be a major part of multiple pathologies, including amyloidosis and neurologic disorders. Current understanding of protein aggregation mainly relies on ensemble measurements and diffraction-limited imaging, effectively averaging heterogeneous aggregation behavior and their kinetics. We introduced real-time photobleaching localization microscopy (REPLOM), a novel approach to super-resolution imaging to observe directly and in real-time heterogeneous aggregation growth. We generated a generic unsupervised machine learning (ML) framework for agnostic, automated protein aggregation analysis concluding what we term an aggregational fingerprint akin to our recent work in Pinholt et al. The aggregational fingerprint contains multiple spatial and kinetic descriptive features for each individual protein aggregate, enabling rapid and precise characterization of aggregation. By combining REPLOM and ML-driven aggregational fingerprinting we were able to track and characterize single aggregates and fibril formation in real-time revealing new heterogeneous growth. We expect our proven combined framework to generalize as a universal, automated protein aggregation analysis platform to drive further mechanistic insights.

**79-Plat**

**Comprehensive fluorescence lifetime fluctuation spectroscopy to investigate protein mobility during aggregation processes in living cells**

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