

Seminar in Computational and Biomedical Imaging

Democratising high-quality live-cell super-resolution microscopy enabled by open-source analytics in ImageJ

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In this talk I will present some of the high-performance open-source approaches we have developed for ImageJ, namely the SRRF and SQUIRREL approaches. SRRF (reads as surf) is a new super-resolution method capable of enabling live-cell nanoscopy with illumination intensities orders of magnitude lower than methods such as SMLM or STED. The capacity of SRRF for low-phototoxicity, allows unprecedented imaging for long acquisition times at resolution equivalent or better than SIM. We demonstrate, using SRRF, live-cell super-resolution images of microtubule, mitochondrial dynamics, the dynamic nanoscale reorganisation of HIV-1 host receptors, as well as extensive cortical actin remodelling during the formation of the immunological T-cell synapse. For the second part of the talk, I will introduce SQUIRREL, an analytical approach that provides quantitative assessment of super-resolution image quality, capable of guiding researchers in optimising imaging parameters. By comparing diffraction-limited images and super-resolution equivalents of the same acquisition volume, this approach generates a quality score and quantitative map of super-resolution defects. To illustrate its broad applicability to super-resolution approaches, we demonstrate how we have used SQUIRREL to optimise several image acquisition and analysis pipelines. Namely, we demonstrate automated benchmarking of different single molecule localisation algorithms on a single dataset; optimisation of DNA-PAINT imaging of clathrin coated pits; cross-validation of single molecule, STED and SIM images of poxviruses; and maximising the quality of single molecule localisation data of neuronal actin rings. By showing the quantitative evolution of data quality over these varied sample preparation, acquisition and super-resolution methods we display the potential of NanoJ-SQUIRREL to guide optimisation of super-resolution imaging parameters.

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