EXTENDED RESOLUTION MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING

Kseniya Korobchevskaya, Marco Fritzsche
Kennedy Institute of Rheumatology (KIR)
University of Oxford
Roosevelt Dr., OX3 7FY, Oxford, UK
E-mail: kseniya.korobchevskaya@kennedy.ox.ac.uk

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1. ABSTRACT

Animal cells continuously sense and respond to external stimuli. Visualising these dynamics remains a major challenge in fluorescence microscopy [1]. Super-resolution total-internal-reflection (TIRF) structural-illumination (SIM) microscopy is perhaps the most promising technology because it offers the right spatio-temporal resolution to image such processes in living cells [1]. However, these new technologies often limit themselves to dedicated equipment. Novel Airyscan advanced imaging was developed to overcome these issues using broadly-available conventional confocal microscopy, utilising a combination of compound detector array, deconvolution, and the pixel-reassignment principles. To quantify the improvement and evaluate its potential for live cell imaging, we performed a detailed study by comparing the Airyscan with conventional confocal and the TIRF-SIM approach. We demonstrated that Airyscan provides spatial resolution as high as confocal at 0.2 Airy Units [3]. In order to judge the potential of both approaches for live cell imaging, we carried out the time sequence imaging of actin filaments in activating Rat Basophilic Leukaemia cells, as they are known for fast and complex dynamics of the actin cortex. We found, that despite Airyscan shows more moderate resolution improvement compared with TIRF-SIM which can provide as high as two folds enhancement, it can produce images with high values of signal-to-noise ratio without increasing the excitation power and acquisition times. This, together with simplicity of the user interface and fault proof default reconstruction settings, makes it a great candidate for live-cell imaging. We envisage Airyscan advanced imaging to overcome the limitations of conventional microscopy and bridge the gap to super-resolution microscopies becoming a major player for quantifying cellular processes at the right spatial and temporal resolution.

2. REFERENCES

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