OPTIMISING SUPER RESOLUTION LIVE CELL IMAGING WITH SIM

Lynne Turnbull

Cell Analysis, GE Healthcare Life Sciences, 32 Philip St, Parramatta NSW 2150, Australia,

Email: Lynne.Turnbull1@ge.com

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The advent of super resolution imaging has pushed the boundaries of how much detail we can see inside cells and in many cases changed the way we think of cellular organization and function. However, the final frontier for super resolution microscopy is imaging live cells. This has proven to be the most challenging aspect of increasing the achievable resolution, with the need to balance cell health, temporal sampling and phototoxicity. Structured illumination microscopy (SIM) has the least light energy input of the three major types of super resolution techniques whilst providing a two-fold increase in resolution in all three dimensions. SIM has three possible implementations – 3D, 2D and in the TIRF plane. Whilst 3D-SIM is the preferred mode due to the increased amount of information gained in all three dimensions, 2D and TIRF-SIM have proved very useful for capturing rapid, dynamic processes with minimal motion artefact and reduced phototoxicity due to the reduced number of raw images required. We will demonstrate the implementation of the 3 modes of SIM in live cell imaging and their target applications, choosing the right fluorophores or fluorescent protein for the sample, and discuss the advantages and disadvantages of each mode with special emphasis on the implementation of SIM imaging microbial cell biology.