USING LATTICE LIGHT-SHEET MICROSCOPY TO IDENTIFY NOVEL CELL SURFACE DYNAMICS

Nicholas Condon, Mark Scott, James Springfield, Jennifer Stow, Adam Wall
Institute for Molecular Biosciences
The University of Queensland
Brisbane, Queensland, Australia
E-mail: n.condon@uq.edu.au

KEY WORDS: Lattice light-sheet microscopy, 4D-imaging, cell biology, membrane dynamics, macropinocytosis, ruffles.

ABSTRACT

The cell surface is an ever-changing environment as the cell responds to different stimuli (in the case of immune cells), migration (cancer cells) or physical forces (epithelial layers). The development of the lattice light-sheet (LLS) microscope by Eric Betzig [1] allowed for rapid acquisitions of 3-dimensional image volumes in under a second, facilitating for the first time near real-time observations of the dynamic behaviours seen at the cell surface.

Macrophages are innate immune cells tasked with surveying their environment for foreign pathogens or debris via a process called macropinocytosis. Using LLS imaging we identified a novel method of macropinocytosis which we called 'Tent pole ruffling' whereby two filopodia-like projections raise a sheet of membrane twisting around one another collapsing back into the cell surface pinching off a macropinosome in the process (Figure 1A)[2]. Cancer cells (MB231) expressing GFP-Lifeact were also imaged for prolonged periods on the LLS, revealing migration behaviours and ruffling types at the leading edge (Figure 1B). We have also employed next generation computing hardware to develop open-source processing and analysis pipelines to analyse and visualise large imaging datasets generated by the LLS.

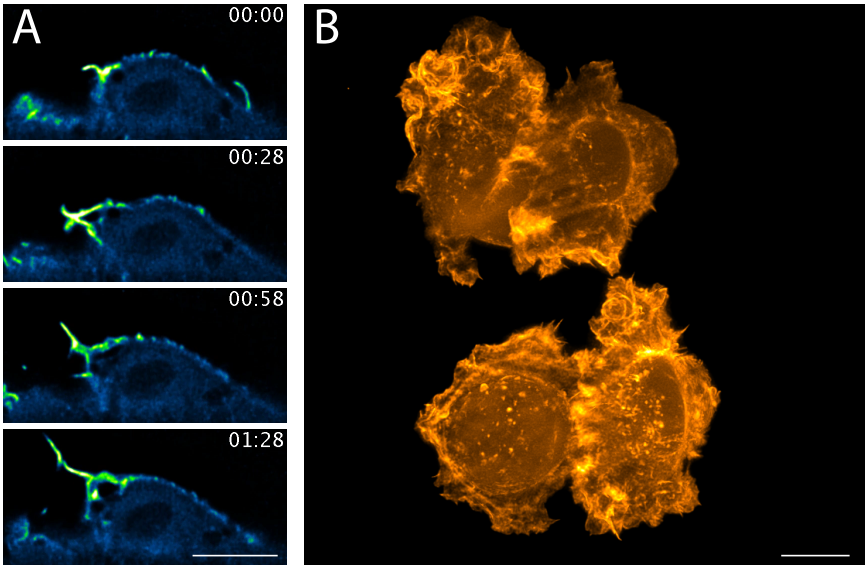

Figure 1 LLS imaging of cellular dynamics with GFP-Lifeact. **A** Single re-slice view in XZ axis of 3D data volumes from multiple time points of macrophages forming tent pole ruffles to form macropinosomes. **B** Deconvolved single maximum projected time point of cancer cells with ruffling boundaries visible. Scale 10 µm.

REFERENCES

[1] Chen et al, "Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution" *Science,* 346; 6208.1257998

[2] Condon et al, "Macropinosome formation by tent pole ruffling in macrophages" *J Cell Biol*, 217: 3873-3885 (2018)