

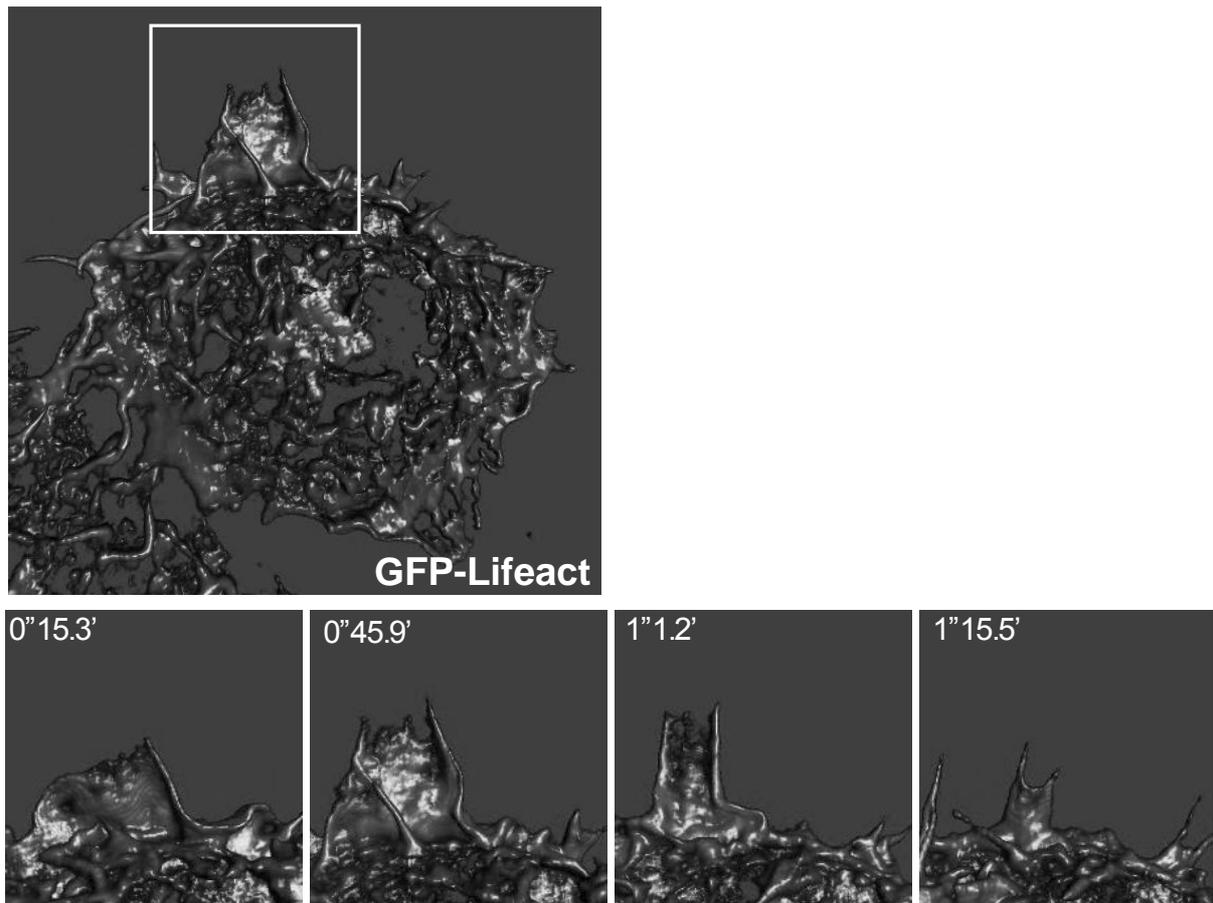
## **Examining macrophage cell surface features using advanced Lattice Lightsheet Microscopy**

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Macrophages are essential innate immune cells with key roles in the surveillance of tissues and in the detection and destruction of pathogens. The macrophage cell surface is geared for these roles by having many protrusions, decorated with sensors and receptors, and by connecting with endocytic and recycling pathways that support high rates of membrane turnover. Plasma membrane ruffles, or more specifically - dorsal ruffles - are prominent features of the macrophage surface, yet they remain poorly understood. Recent findings suggest that ruffles play key roles in pathogen detection and activation of macrophages by housing Toll-like receptors and signalling molecules. Dorsal ruffles close to form macropinosomes or phagosomes for the uptake of fluid, particles and pathogens.

This project aimed to characterise dorsal ruffles through the application of advanced live cell imaging, including the first application of lattice lightsheet imaging to study dorsal ruffles and macropinosomes in activated macrophages. We have identified a novel mechanism for ruffle formation using lattice lightsheet imaging (figure 1), and performed functional characterisation of ruffle associated proteins. Additionally, a number of image-based quantification tools were developed and used as part of this project to analyse large data sets of images for semi-high throughput analysis of different macrophage functions, including assaying ruffle formation, protein enrichment in dorsal ruffles and downstream ruffle related events including, macropinocytosis and phagocytosis. The outcomes reveal new, important morphological and functional features of surface ruffles and provide new imaging and image analysis strategies for high resolution, big image live data.



**Figure 1 Macrophage cell surface features viewed via Lattice lightsheet microscopy.**

Timelapse image sequence of GFP-LifeAct expressing RAW264.7 macrophages captured on a Lattice Lightsheet microscope at Janelia Farm Research Campus, rendered for visualisation using Arivis Vision4D.

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