

Live imaging of human neutrophil using a multimodal imaging approach
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Neutrophils are key immune cells which defend against *Staphylococcus aureus*, a formidable pathogen that causes wide range of infections. When ingested by neutrophils, *S. aureus* becomes contained in a compartment known as phagosome. Inside the phagosome, mechanisms such as acidification occurs in attempt to kill *S. aureus*. Despite various killing mechanisms, some *S. aureus* are able to resist neutrophil killing and survive inside the phagosome, ultimately causing neutrophils rupture and cell death. This allow *S. aureus* to escape from neutrophils and eventually leads to infection elsewhere. A better understanding of neutrophil-*S. aureus* interaction will allow us to develop new therapies for *S. aureus* infections.

Imaging human neutrophils is challenging due to their light sensitivity. We therefore developed a multimodal imaging platform to perform long-term live imaging of neutrophils and *S. aureus* [1]. This allows us to switch between low light low resolution and higher intensity higher resolution modalities. Low light modalities are used to reduce phototoxicity, enabling visualisation of early events with minimal invasion. Higher resolution is used at specific timepoints to investigate neutrophil and *S. aureus* interactions at subcellular level.

We implemented three colour brightfield, confocal and novel form of multifocal structured illumination microscopy in 3D. Phagosomes containing *S. aureus* acidifies to facilitate the killing of bacteria. Interestingly, we observed a mixed of acidified and non-acidified intracellular *S. aureus* present in neutrophils when live GFP-*S. aureus* labelled with pHrodo was used. Additionally, cell walls were observed in non-acidified intracellular bacteria using live structured illumination, suggesting that non-acidified *S. aureus* may be undergoing cell division (Figure 1).

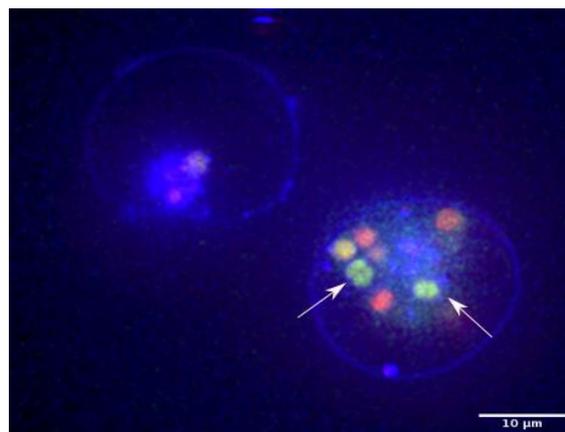


Figure 1. Acidified (red) and non-acidified (green) GFP-*S. aureus* labelled with pHrodo in neutrophils. Cell walls were observed in non-acidified bacteria (white arrows).

[1] Liyana Valiya Peedikakkal, Andrew Furley, Ashley J. Cadby .2018. A Multimodal Adaptive Super-Resolution and Confocal Microscope <https://doi.org/10.1101/397273>