# Development of FLIM system to understand the microenvironment of human immune cells

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*Staphylococcus aureus* is a highly versatile bacteria that causes wide range of infections, and treatment is increasingly difficult due to its extensive antibiotic resistance profile. Neutrophils are key immune cells to defend against *S. aureus*, where they engulf and contain *S. aureus* in a compartment known as phagosome. Factors such as pH level in neutrophil phagosome changes to facilitate killing of *S. aureus*, yet *S. aureus* can evade neutrophil-mediated killing. Visualising the phagosome microenvironment is challenging due to the highly autofluorescent nature of neutrophils. Here, we show the development and use of time-correlated single-photon counting (TCSPC) to perform fluorescence lifetime imaging microscopy (FLIM) in human neutrophils. The lifetime of fluorescently-labelled *S. aureus* can be used as a functional readout of the microenvironmental changes in neutrophil. We aim to use this technique to understand the pH changes of neutrophil phagosome in presence of *S. aureus*, and to correlate the change of pH in the phagosome to bacterial killing.