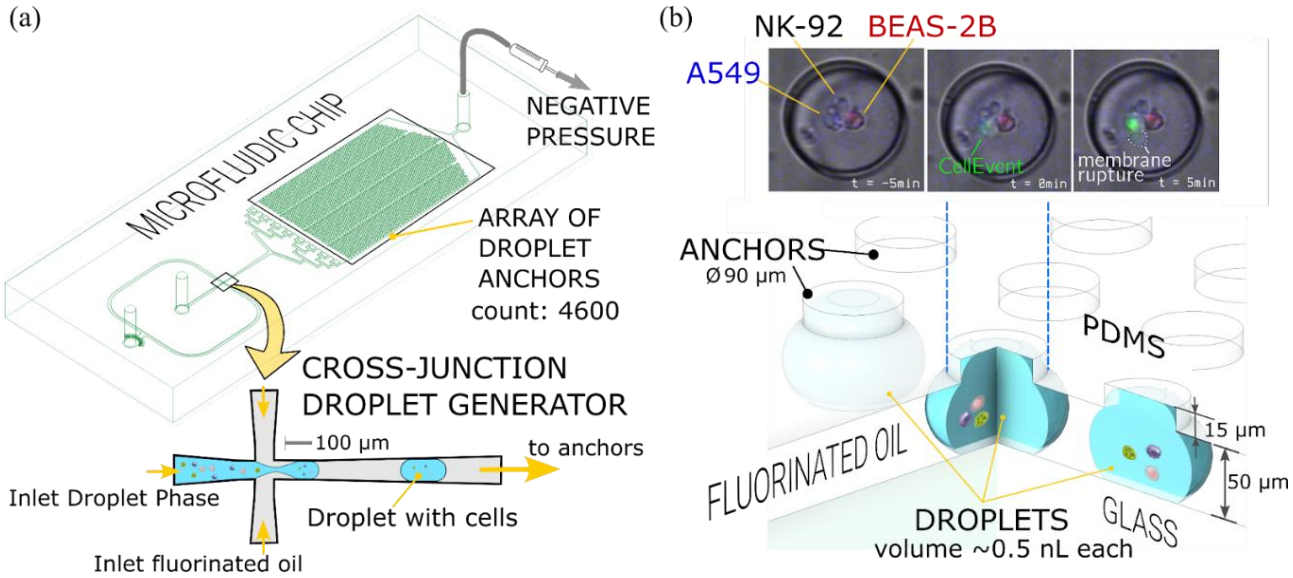
Inflammatory modes of regulated cell death in respiratory viral infections

Infected cells employ various strategies to limit viral spread. After detecting the virus, cells can secrete interferons to inform neighboring cells about infection, giving them time to prepare. They may attempt to digest viral material through autophagy or sacrifice themselves, triggering apoptosis or so-called inflammatory cell death such as pyroptosis or necroptosis – both associated with cell membrane rupture. Cell death can also be triggered by natural killer (NK) cells, which recognize and eliminate infected or cancer cells. Viruses often employ their nonstructural proteins to oppose autophagy and apoptosis and have acquired the capability to hijack and subvert these processes for their benefit. The complex interactions between the host cell and the virus may eventually lead to inflammatory cell death.

Inflammatory modes of cell death have become relatively well understood in isolation, however, their mutual interactions and interactions with apoptosis, which ultimately dictate cell fate, are not sufficiently understood. The distinction between death types is essential as inflammatory death pathways have an immunogenic potential and may trigger effective adaptive immune responses, but also harmful systemic inflammation.

In the project, we will use two respiratory viruses, RSV and Influenza A, and a panel of human respiratory epithelial cells to investigate the virus–host cell and NK cell–infected cell interactions that lead to divergent types of cell deaths: non-inflammatory and inflammatory. Our aim is to elucidate crosstalk between apoptosis and inflammatory pathways both at the single-cell level and at the level of the infected population. We will investigate what factors determine cell fate decisions and dictate the proportion of cells exhibiting a given death type. We will also verify whether the mentioned deaths are executed exclusively, or whether a single cell may exhibit a combination of apoptosis and immunogenic death pathways.

We will use single-cell techniques, including live microscopy, allowing for the observation of different death types in single cells of the infected population. The cell-to-cell interactions will be investigated with the help of droplet-based microfluidics, which enables performing thousands of concurrent experiments in isolated volumes (as in the figure below). We will use machine learning-based analysis of live microscopy images to discern specific types of cell death based on cell morphology and fluorescent markers. To verify the consistency of our findings and provide a broader picture of regulatory mechanisms, we will construct mathematical models of the death pathways crosstalk at the single-cell and cell-population levels. This research, performed in collaboration with a world-leading specialist on pyroptosis, Prof. David Brough, from the University of Manchester, will enable better planning of antiviral therapies by providing a step-change in our current understanding of the fundamental processes controlling cell death pathways.



The microfluidic system allows for immobilizing more than 4000 droplets for experiments studying interactions of droplet-encapsulated cells. (a) Schematic view of the design of our device. (b) Top: selected snapshots of the droplet containing three cells: NK-92 (gray - no staining), noncancerous BEAS-2B (stained red), and cancerous A549 (stained dark blue), showing the death of the cancerous cell induced by NK-92 cell. The lighting of CellEvent (green) informs about the cell death initiation, which is followed by cell membrane rupture, suggesting pyroptotic cell death. (b) Bottom: the view of the droplet anchored in the well.