COVID research on Octopus during the pandemic

Early in the pandemic it was recognised that the CLF, and in particular Octopus, had the potential to assist with research into SARS-CoV-2 and COVID-19. Having shut down normal operations, the facility was able to issue a rapid call for research proposals relevant to COVID-19. This call was open to researchers worldwide, from both academia and industry. Given the urgency of the situation, a light touch review process was used, with a brief scientific case for support being assessed by two members of the usual Facility Access Panel. A number of successful proposals were received, and the research was done by members of the Octopus team, working under stringent COVID control measures and in close contact with the researchers. Brief descriptions of the projects are below.

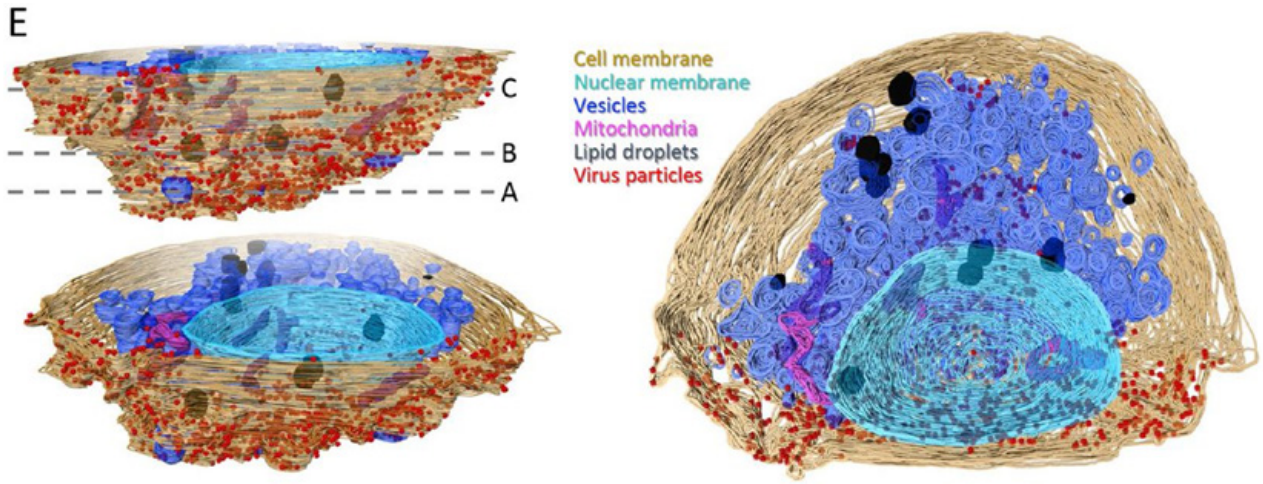
These projects demonstrate how the CLF is able to respond quickly to new challenges, and show the potential of Octopus for research into infection and immunity, an extremely important area for society. We believe this will be a growth area for the facility in years to come.

Cryo "slice and view" scanning electron microscopy to look at the location of SARS-CoV-2 virus in cells

eBIC/Diamond – Oxford – Cambridge – Pittsburgh – CLF (Benji Bateman, Marisa Martin-Fernandez, Laura Zanetti Domingues)

The objective of this project was to obtain a high resolution 3D map of the location of the virus within the cells of patients, to discover more about infection mechanisms and possibly give clues as to how the infection could be treated. Using cryogenically-preserved rather than chemically-fixed samples meant we were closer to the native state of the cells.

3D data sets were obtained which, after a significant amount of intensive manual data processing, revealed the location of the virus particles in the cells. The data were correlated with cryo-electron tomography data from eBIC to provide a detailed structural and ultrastructural picture of the virus's replication cycle, for example showing extensive membrane tunnels formed for the virus to exit, and lesions near the exit region of the cell. The work was subsequently published in *Nature Communications* (Mendonça, L., Howe, A., Gilchrist, J.B. *et al*. Correlative multi-scale cryo-imaging unveils SARS-CoV-2 assembly and egress. *Nat Commun* **12**, 4629 (2021). https://doi.org/10.1038/s41467-021-24887-y).



Surface rendering of the segmented volume of a SARS-CoV-2-infected cell. Segmented organelles and virus particles are labelled with the colours indicated (Howe et al., Nature Communication 2021).

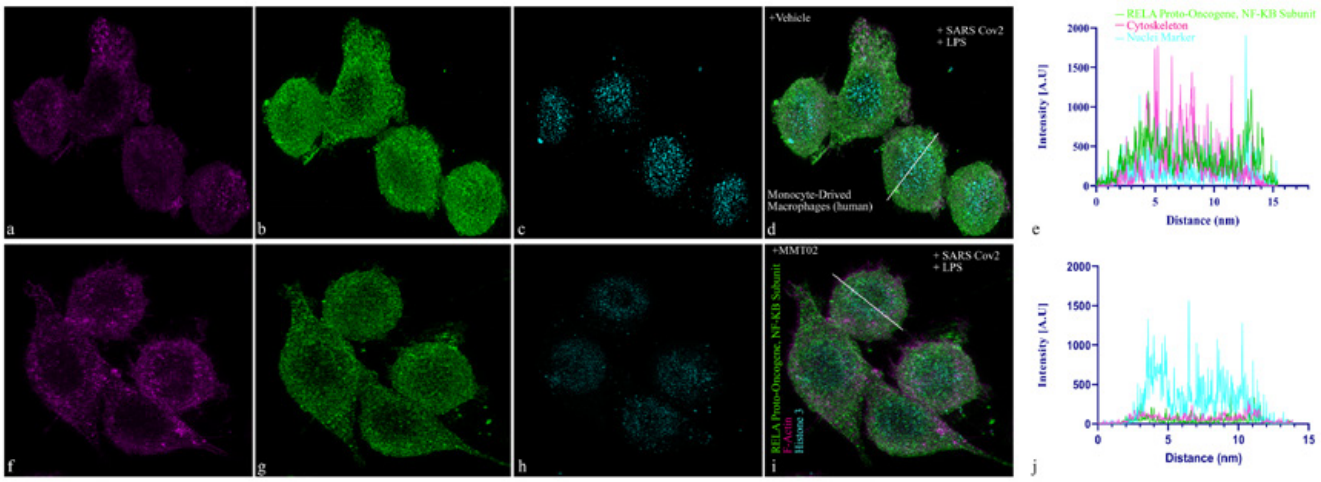
Investigating the action of drugs designed to modulate the immune response

Metamorph Therapeutics – CLF (Stan Botchway)

Acute Respiratory Distress Syndrome (ARDS) is a form of inflammation that results from an over-reaction of the immune system, and is a common cause of serious illness and death in COVID-19. This project used super-resolution microscopy to investigate the effects of a drug candidate molecule designed to "turn down" the immune system and reduce inflammation.

Sample preparation for this project was challenging, and was a good example of the difficulties that occur with remote working, which makes the regular interaction between user and CLF link scientists more difficult.

After a number of iterations of sample preparation, good images were obtained that show the reduced expression of inflammatory markers in response to the treatment.

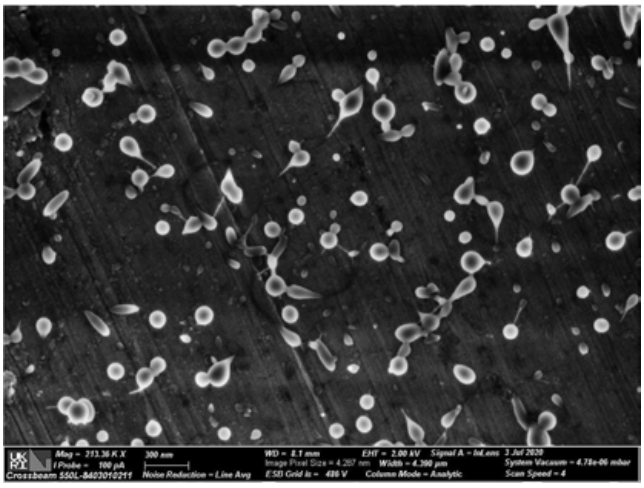


STED nanoscopy results showing reduced number localization of RELA with MMT02 treatment compared to a vehicle control in macrophages challenged with LPS and the novel Coronavirus SARS Cov2

Looking at systems to deliver antiviral drugs to the brain

Biocyto – CLF (Benji Bateman, Laura Zanetti Domingues)

Doctors have recorded a number of life-threatening brain related symptoms of COVID-19 infection, such as stroke and inflammation that have been recorded in about 30 – 40% of cases. Recent studies have found the novel coronavirus in the brains of fatal cases of COVID-19 and it is plausible that this is causing neurological disorders by directly infecting the brain, or as a result of the strong activation of the immune system. Neural and immune cells can serve as reservoirs of latent coronavirus. The blood-brain barrier makes it difficult to deliver drugs to the brain, and Biocyto have developed nanoparticle drug delivery platforms to overcome this problem. They used high-resolution SEM at Octopus to characterise the nanoparticles for size and uniformity. Large numbers of particles were screened, with an example image shown.



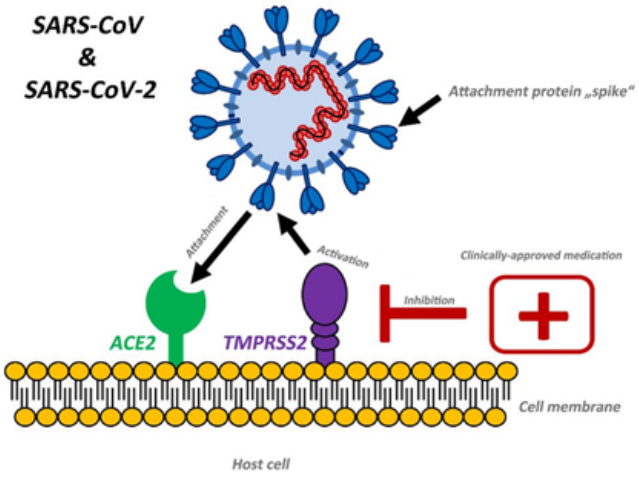
Studying the interaction between SARS-CoV-2 spike protein and its target receptor

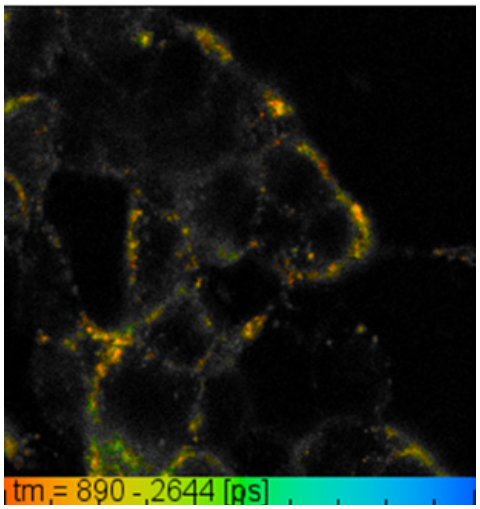
Biocyto – CLF (Benji Bateman, Laura Zanetti Domingues)

The SARS-CoV-2 virus infects cells through binding of the spike protein on its coat to the ACE2 receptor molecule on cells (left). This project used a technique called Förster Resonance Energy Transfer (FRET) with Fluorescence Lifetime Imaging (FLIM) to study the interaction between the spike protein and its target.

FRET-FLIM is able to show the level and location of molecular interactions in the cell. In the image (right), short fluorescence lifetimes, which show interactions between ACE2 and the spike protein, appear orange.

The method was used to image cells treated with drugs: Losartan, which is known to block interactions of the receptor; and CBD, a drug that modifies membranes. FRET-FLIM allowed us to measure whether these drugs interfere with binding of the virus to its target.





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