Cryo-Lamella preparation to visualise host pathogen interactions by CryoET

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CryoET is a powerful method capable of determining protein structures at subnanometer resolutions in situ. Although some biological specimens are small enough (<300 nm thick) to be visualised by cryoTEM, many are too big and require additional sample preparation. Specimens larger than 300 nm include eukaryotic cells, bacteria biofilms, and most bacteria. Therefore, to investigate host-pathogen interactions using cryoET we need methods to create thin samples containing features of interest.

Cryo-FIB-SEM is a method for generating a thin (<200 nm thick) cryo-lamella by milling away unwanted sample with a focused ion beam. Despite considerable technological developments, several elements of this workflow remain challenging, such as the vitrification of thick samples, localisation of targets within thick samples, and automation. Here we explore the use of high pressure freezing, for the vitrification of thick samples; cryo-light microscopy, to localise region of interest and target cryo-lamella preparation; and milling procedures, to create an optimized workflow for faster cryo-lamella preparation. We utilise these methods to visualise host pathogen interactions by cryoET

The development of cryo-lamella preparation techniques underpins the utility of cryoET and in situ structural biology and broadens its target range and diversity.