ATOMIC FORCE MICROSCOPY AND FLUORESCENCE MICROSCOPY OF MALARIAL HEPATOCYTES

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Malaria is a devastating disease which kills millions of people around the world each year. The disease is caused by one of several parasites of the genus *Plasmodium*. The parasite enters the human host in the form of sporozoites, delivered by a mosquito bite and reaches the liver through the bloodstream. In the liver, the parasite undergoes a development and multiplication process, after which thousands of merozoites, a second form of the parasite, are released into the blood stream, where the characteristic fevers of the disease occur. Because the liver stage of infection is asymptomatic, and samples are more difficult to extract, it has been studied much less than the blood infection stage, but is vital for the lifecycle of *Plasmodium*, and for the progression and severity of the disease[1].

We have used simultaneous atomic force microscopy (AFM) and optical microscopy to study a human hepatoma cell line, Huh7, infected by *Plasmodium berghei*. Exposure of liver cells to the parasites causes a low percentage of infection (ca. 1%) of the cells, and so we used *P. berghei* genetically modified to express green fluorescence protein (GFP). This allowed fluorescence microscopy to be used to identify and locate infected cells. Having identified which cells were infected, we studied both infected and uninfected cells using AFM to identify any morphological differences upon malaria infection.

We studied both fixed cells and living cells under physiological conditions, in order to ensure the results were not artefacts of the drying procedure. The results show considerable and significant differences between infected and uninfected hepatocytes. The AFM could clearly differentiate the infected from the uninfected cells, and the infected cells showed characteristic features which suggest interesting possibilities about the occupation of host hepatocytes by the *Plasmodium* parasites.

A further batch of cells was subjected to a proteolysis treatment. This process removes the surface proteins on the membrane of the cells, enabling us to eliminate morphological features associated with these proteins. These results show how the presence of these proteins affectthe cell-surface features observed in the infected hepatocytes.

References:

1. Prudencio, M.; Rodriguez, A.; Mota, M. M.,. *Nature Reviews Microbiology **11*** (**2006)**, 849.

Figures:

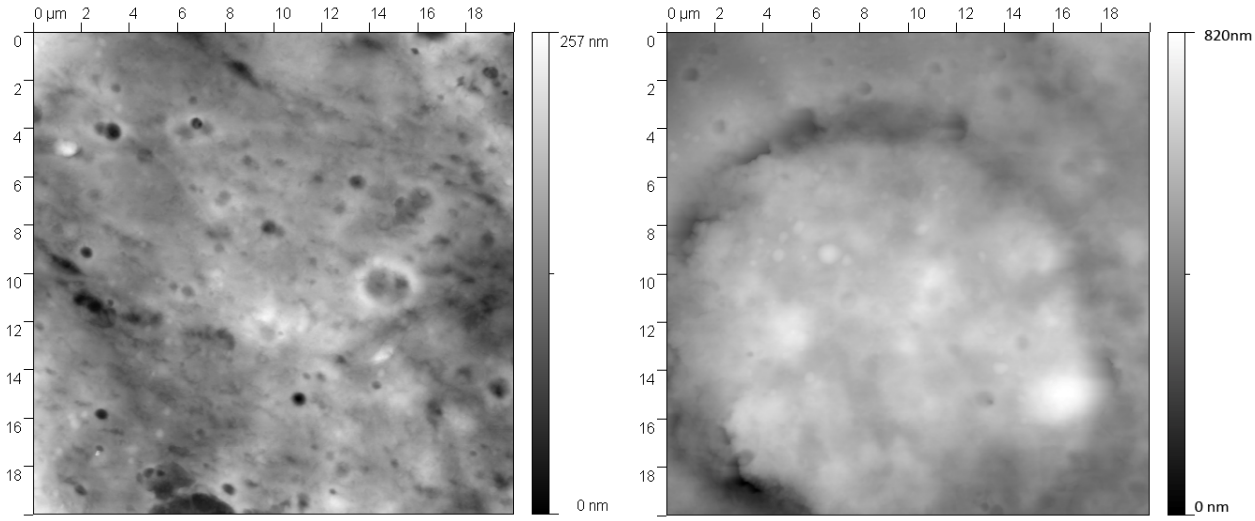

Figure 1: Tapping mode AFM images of an uninfected hepatocyte (left), and a hepatocyte infected with malaria (right).

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