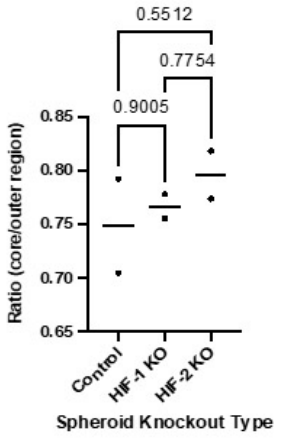

Figure 4. Portion of hypoxic core cellular content normalised to the outer region shows no significance with only 2 spheroids per group.

increased sample size turn out to be a more robust measure, but here there was no significance.

Using the higher magnification micrographs, I also compared the length of the mitochondria in each of the spheroid knockout types (Figure 5). As expected, (as the mitochondria are sectioned tubes) I did not see any significance between spheroids or groups indicating there was no difference in cellular orientation/alignment induced by knockout.

From this initial pilot study, I can continue the analysis on a complete data set and look specifically for intercellular connections, quantify cristae and likely changes in the ER. Further, we also high pressure froze the samples for mass spectrometry imagining (3D-OrbiSIMS) so watch this space!

What did I learn?

I learnt to resin embed my spheroids (post osmium steps). I was trained (using autophagosomes for a

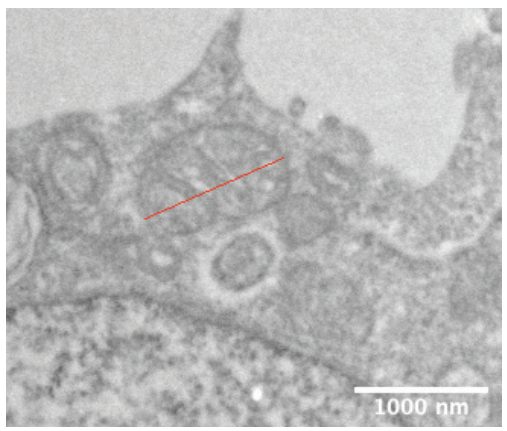

Figure 5. A high magnification image of a mitochondrion. The red line shows how I measured each single mitochondrion for analysis.

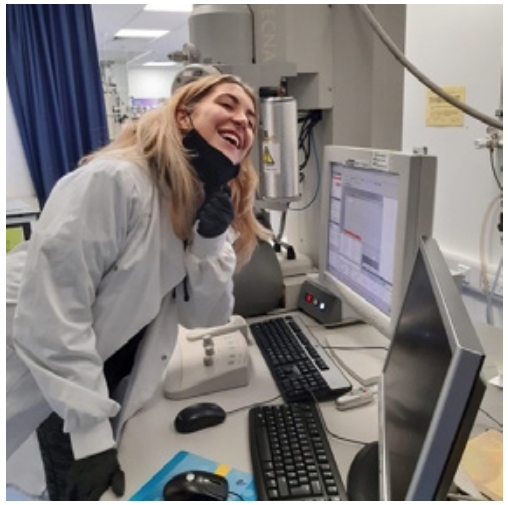

Figure 6. Me next to the Tecnai-12, which I used for my imaging.

related project) to use the Tecnai-12 independently by Dr Julie Watts. I also was able to spend some time on a scanning electron microscope (Zeiss 550 crossbeam) in transmission mode, with Dr Jacqueline Hicks. From this I was able to use the images for comparative microscopy, looking especially at the resolution (or not) of double membranes in the nucleus. I had a great time and managed to join in with many of the general processes within the *nanoscale and microscale research centre*.

I also had an opportunity to present my work to others at different stages of the studentship. This allowed me to grow my confidence in presenting, particularly with this type of data.

How has this affected my long-term goals

From undertaking this summer studentship, I was able to confirm that I would like to have a future career which includes imaging on a nanoscale. Halfway through this placement I decided to transfer from BSc to MSci version of my degree, This summer studentship was key in securing me a place on the MSci, as I gained both lab experience and became confident that I would enjoy the extra year of the course. The MSci includes a nine-month placement in either academia or industry, I am hoping to have a placement which has multiple microscopy elements.

Synchrotron X-ray Nanoprobe Analysis of Archaeological Samples: A Feasibility Study

Student: Jack Pearce
Supervisor: Julia Parker (Diamond Light Source) with Anita Radini (University of York)
Project location: Diamond Light Source and University of York

The summer studentship carried out jointly with Diamond Light Source and the University of York aimed to explore and test the use of synchrotron X-ray Nanoprobe analyses for the study of archaeological remains.

Introduction

Diamond Light Source is the UK's national synchrotron facility. Synchrotrons accelerate electrons in a storage ring where they emit radiation (from UV to X-rays wavelengths). Each beamline uses a specialised setup, energy range, resolution and technique to analyse the internal makeup of a sample, for example X-ray diffraction (XRD) or spectroscopy. Beamline I14 is a Hard X-ray Nanoprobe beamline using X-ray fluorescence (XRF), diffraction and imaging techniques for mapping elemental composition, and structural variations with 50nm resolution [3].

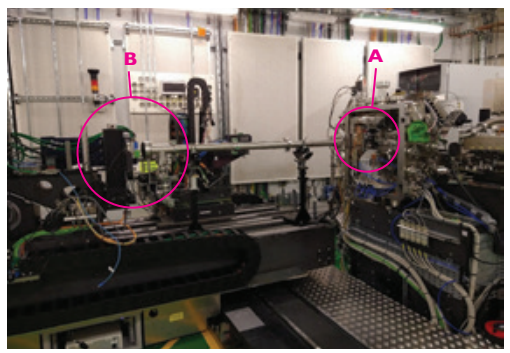

Figure 1. The I14 beamline experimental hutch showing the endstation where the sample was mounted (A) and detector (B).

Analytical techniques used in archaeology and bioarchaeology include XRD, Raman spectroscopy and electron microscopy to study various aspects of historical samples. XRF has been used in various other studies in archaeology such as by analysing pottery fragments to study their composition [1], artefacts such as historic astrolabe [2] and to assist in criminal investigations through the analysis of dental resins [4]. Each of these techniques has its own unique set of advantages and disadvantages, including ease of sample preparation, achievable resolution, and field of view. In this work we have used nano- XRF in order to give a high resolution map of the elemental composition of the samples, and aimed to test the feasibility of preparing suitable samples, their integrity during X-ray exposure and develop knowledge of methods for subsequent data processing and analysis.

Methods

The samples were prepared for analysis using a gallium FIB, milled to a size of 20 x 10 x 0.5 microns mounted onto Copper Onmiprobe grids. The thickness of the samples was chosen to give a good balance between penetration of the X-rays, signal strength and not to compromise the resolution of the X-ray probe.

Grids were mounted on beamline sample holders and placed in the endstation (see Figure 1) and scanned through the focussed 12 and 15 keV X-ray beam with 50 nm steps and a 0.015 sec/pt dwell time. These conditions provided a high-resolution image

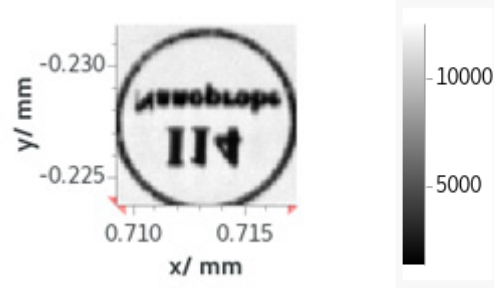

Figure 2. W la map from a 1micron thick W tungsten patterned calibration chart.

of the samples. Data were analysed using PyMCA to extract individual elemental maps.

Results

Data from the archaeological sample tested are not presented here as they will be published elsewhere, however, the samples were successfully able to be FIB milled and analysed to extract meaningful nanoscale elemental composition maps. Instead, the results from some calibration samples are shown in order to demonstrate the application of the technique.

The data from XRF can be viewed as a map of the sample as shown in Figure 2. This clearly shows the outline of the logo where the tungsten is located. Figure 3 shows an example of XRF data plotted in a spectra. From this spectra the fluorescence line for the elements Ti and Cr are visible, from this the composition of the sample can be observed.

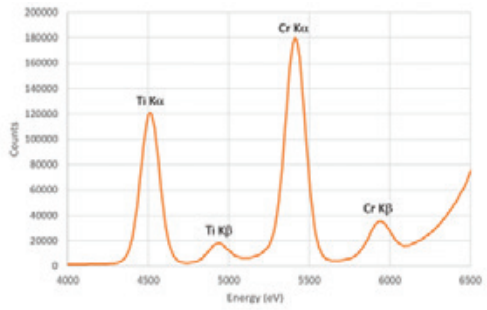

Figure 3. Example nano-XRF spectra from a sample containing Chromium and Titanium with the fluorescence lines labelled.

Conclusions

In conclusion, the nanoprobe XRF mapping has proven to be highly effective in the analysis of the specimens. The technique can provide an insight into the nanoscale elemental composition of the specimens. With these irreplaceable samples, preservation is key and as the technique is virtually non-destructive, it allows minimal damage to occur to the samples which aids in the preservation and further study of them.

The experience at Diamond allowed me the opportunity to visit a dedicated research facility to collect data which provided me with a valuable insight into real life microscopy experiments. I thoroughly enjoyed my time visiting Diamond and every day was filled with the chance to get real life data on state of the art equipment going towards the group's research. I was privileged to work with a great research team on an incredibly interesting piece of research and I am hoping to go on to study a PhD using microscopy techniques, such as electron microscopy, so I can work in further research groups.

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Acknowledgements

Data were collected at Diamond Light Source, Beamline 114 under proposal number MG27407

Endomembrane interactions in microsporidian infection

Student: Mostin Hu
Supervisor: Dr John Lucocq
Project location: School of Medicine, University of St Andrews

Lay summary:

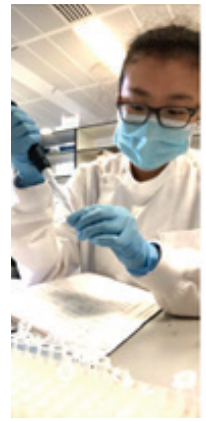
Microsporidia is a group of intracellular eukaryote parasites which require a host cell to survive and reproduce. For most people these organisms do not cause harm, but infection in people with a compromised immune system (for example, due to chemotherapy or HIV-AIDS) can cause serious illness and even death. We previously identified close association of host membranes with microsporidia and speculated that these membranes help wall off and protect the parasite. This project used microscopical techniques and knockdown of host proteins to characterise these membranes and potential mechanisms.

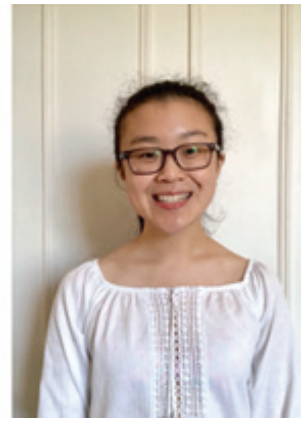
Project Aims:

Previous research in the Lucocq lab demonstrated a host-endomembrane response is triggered by microsporidia-infected cells. This project aimed to investigate which membranes of the are recruited and whether this response is specific for proteins of the organism. By suppression of host cell components, we aimed to elucidate whether this response was protective to the host or beneficial for the parasites.

What I learned:

I am so incredibly thankful to have been given this summer studentship as it allowed me to not only develop key laboratory skills, but it also allowed me to learn and practice the qualitative and quantitative analysis of microscopy images. I was able to refine key research and laboratory techniques such as sterile cell culture, Western blotting, IF staining and how to choose antibodies against the proteins of interest using known data available from other similar species and performing sequence alignment. Working primarily with immunofluorescence microscopy, I learned how to obtain images using systematic





uniform random (SUR) sampling, a method which allows for the non-biased acquisition of images imperative for quantification.

How this project has affected my long-term goals:

This summer studentship has offered me the time and experience to confirm my interest in cell biology research. As a medical student, I found that research is often portrayed to be a separate career path from clinical medicine; however, I have grown to realise that both fields are highly complementary to one another. Crucially, I have been able to appreciate the many transferable skills that can be learned through research which will hopefully make me a better clinician. The skills of inquiry, creative thinking, hypothesis testing, pattern recognition, and critical analysis of data which are practiced daily in research are equally important in clinical practice.

I am very keen to undertake a PhD during my medical training in the field of host-pathogen interactions, an interest I developed through this studentship and hope to use the microscopy techniques I learned this summer in my future research.