

Scholar Report

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Title of Scholarship Project:	Unveiling the Metabolic Tango: Exploring the Interplay of Lipid Droplets and Mitochondria Through a New Staining Technique

1. My Research Project

My project is about studying lipid-mitochondrial interactions. To understand these interactions, I developed a new co-staining technique that would combine the use of Oil red O and Mitochondrial Trackers to allow the visualisation of these interactions in patient tissue. By applying this new technique to patients, it could help to understand how factors such as age and BMI are involved in the pathology of cardiovascular and metabolic diseases such as diabetes.

I learnt a lot about problem-solving, adaptability and resilience whilst facing multiple setbacks and challenges throughout my project. Overall, my time working on this project has been incredibly rewarding and has set me forward to a promising research career.

1.1 Research Undertaken

To investigate the changes in lipid-mitochondria interaction in the heart, patient samples were collected during routine heart surgeries at the Leeds General Infirmary. Samples were immediately embedded in OCT, a cryoprotectant and stored in -80°C for later use. I specifically selected each patient sample for my research based on the key criteria of sex, age, and BMI. I first perfected my cryosectioning technique using mouse skeletal muscle samples before moving to human patient samples. After selecting 5 female patients, these samples could be cut using the

Leica CM3050 S Cryostat at -20°C into sections of 10µm onto slides. Slides were air-dried for 30 minutes before storage in -70°C.

Patient	Age	BMI	Health Conditions											
A		53	30.5	Degenerative Aortic Stenosis										
B		39	26.8	Endocarditis, smoker										
C		40	23.9	Endocarditis, AV replacement, smoker, asthma										
D		67	32.9	Aortic Stenosis, Fatty liver & cirrhosis, Liver transplant, Diabetes, Hypertension, Hypercholesterolaemia, Varicose Veins										
E		74	37.6	CKD stage 3, Diabetes, Pacemaker										

Table 1. Patient Information for the samples selected.

Alongside cryosectioning, I also worked on stain preparation. I used some of the practice heart sections I collected while refining my technique. The first stain I used was H&E staining; a stain used in diagnostics and research to differentially colour the cell wall and nucleus. I followed the widely used protocol: Hematoxylin for 1 minute, cold water rinse, 3 dips in 0.25% HCL in ethanol, hot water for 2 minutes, Eosin for 1 minute, cold water rinse and dehydrated using different concentrations of ethanol. Cover slips were mounted with DPX mounting media.

To visualise lipids, I used Oil red O, a well-known dye that is usable in non-living tissue. The first protocol that I had attempted was unsuccessful, so I researched 3 other protocols and consulted my supervisor. Due to my earlier results lacking any visible red and being very dark blue, I pursued a protocol with an extended time in Oil red O and a reduced time in Hematoxylin. Finally, I developed a suitable protocol: fixation with 4% PFA for 10 minutes, 2 minutes with propylene glycol, 30 minutes with 60°C Oil red O, PBS rinse, 30 seconds with Gills Hematoxylin.

My third staining technique was the most complicated as it has never been done on fixed tissues and is a fluorescent stain. I trialled 2 different fluorescent stains Mitotracker and MitoBrilliant. Both stains have only been used in staining live cell cultures but the slides I was using were non-living. To prepare this technique, I researched a range of current protocols for living cells and the ways to adapt them for my method. I ran my first protocol by my supervisor, and we decided to start with a higher concentration of MitoTracker and a standard concentration of MitoBrilliant then work our way backwards to more specific staining. After multiple repeats through trial and error, I found that my method involving a 200nM solution of MitoBrilliant was most effective. The full protocol was as follows. Fixation with 4% PFA for 10 minutes, 3 minutes with PBS, 10 minutes with 0.2% Triton-X, 3 minutes

with PBS, 30 minutes incubation of 200nM of MitoBrilliant at 37°C, 3 minutes with PBS and mounting with VectaShield fluorescent mounting medium.

After this I combined the Oil red O and MitoBrilliant protocols to develop the co-staining technique that would allow me to view lipid droplets in relation to mitochondria, this involved steps 1-6 of MitoBrilliant staining followed by steps 2-5 of Oil red O staining. Slides were imaged using the Zeiss X10 light microscope and EVOS microscope using the Cy5 filter at a range of 662nm and analysed using Zen.

I received 2 wells of spare human female ventricular fibroblasts which had been cultured by a PhD-student at LICAMM. I used these cells to act as a comparison to my pre-fixed tissue and followed protocols for MitoBrilliant and Oil red O stain for both wells. The MitoBrilliant did not work as expected and due to limited cell cultures could not be repeated. The oil red O stain worked on live cells.

Once cryosectioning of all of the patient samples was complete they could be stained with all 4 methods. H&E staining was used to assess the quality of tissue and confirm any damage. During cryosectioning, some samples showed signs of visible damage including structural damage such as cracks and water damage potentially due to issues when they were originally frozen. This means that results may not be as reliable.

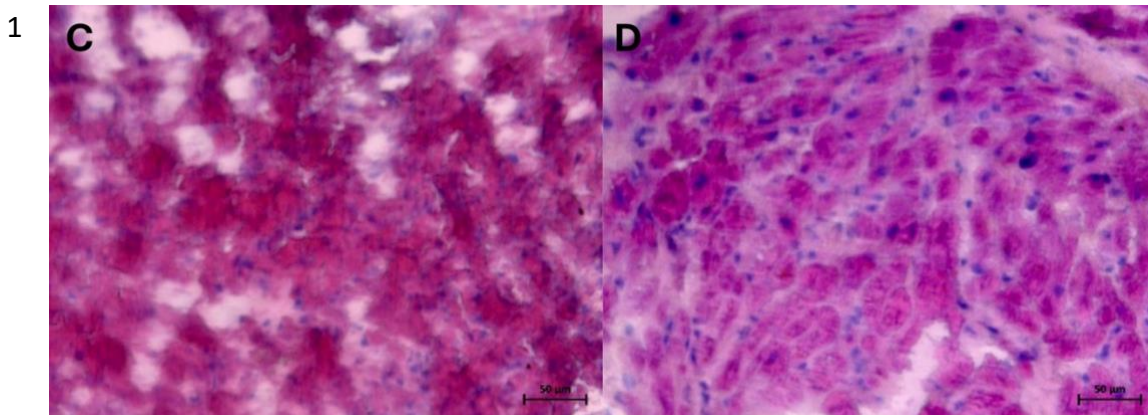


Figure 1: H&E-stained sections from patients C and D. C shows structural damage in comparison to D.

Images collected from MitoBrilliant staining show that staining was successful.

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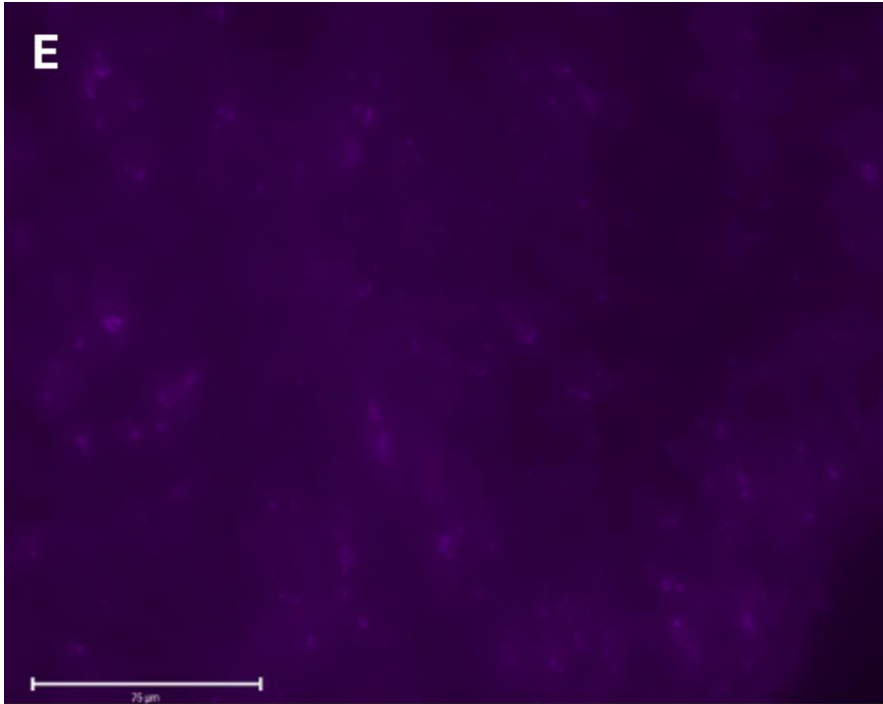


Figure 2: x400 magnification of MitoBrilliant staining in patient E.

It would be recommended to image as soon as possible to minimise photobleaching.

Oil red O staining was successful in both living and non-living tissue. Images taken from patient samples demonstrate a higher level of lipids in patients C, D and E.

Images taken of the cultured cells show some differences such as smaller and less clustered lipid droplets in comparison to patient samples.

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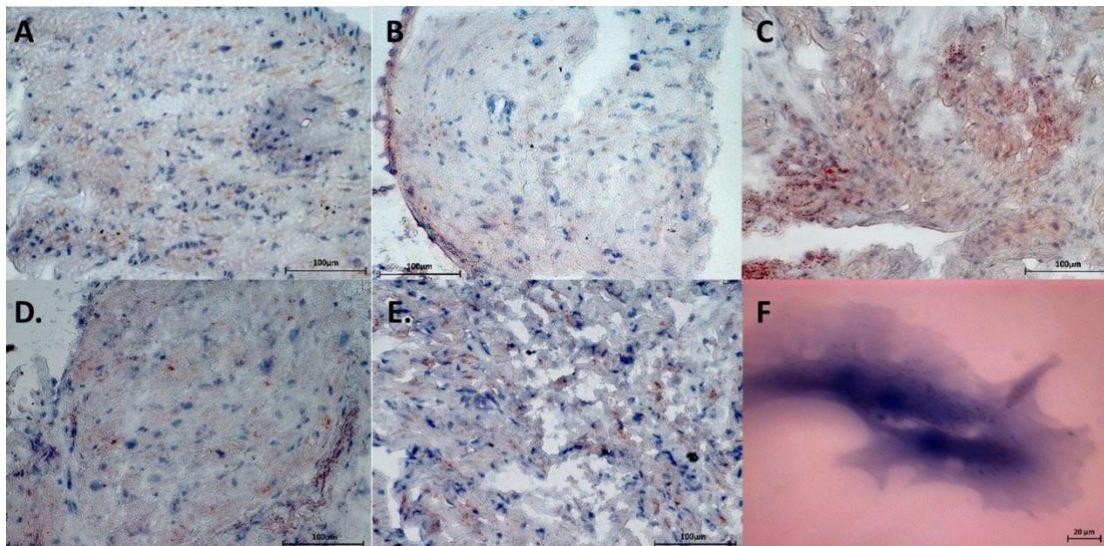


Figure 3: images of Oil red O stain on both patient samples A-E and on cultured cells in F.

Images from the fluorescent view of the co-stain were edited to allow only mitochondrial staining to be visible, then were overlaid on the images from the

bright-field view of the microscope which displayed Oil red O staining. I marked each lipid droplet then compared this to areas of mitochondrial staining to count the lipid droplets in contact with mitochondria and how many were not.

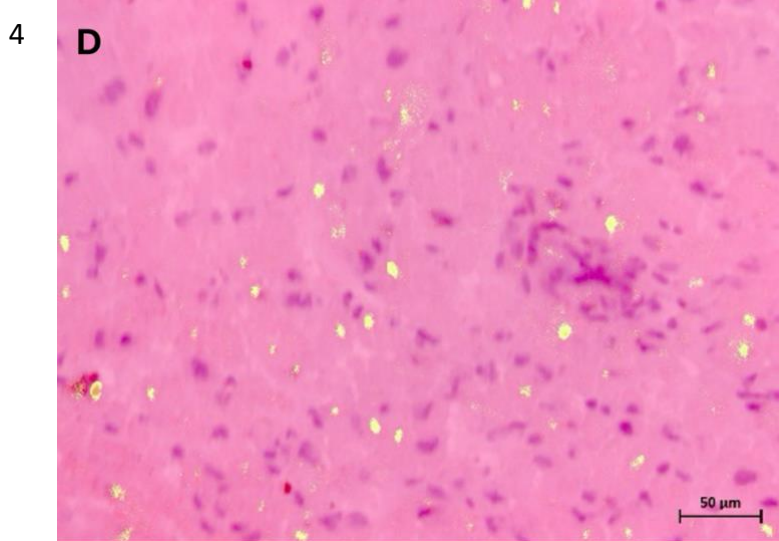


Figure 4: An image of the successful co-stain on patient D.

A linear regression between age and BMI of patients was conducted to find a significant relationship. This led me to conduct a multiple linear regression which compared the percentage of mitochondrial interactions and BMI but accounted for the co-variable of age. The result was a poor linear fit likely due to a small sample size.

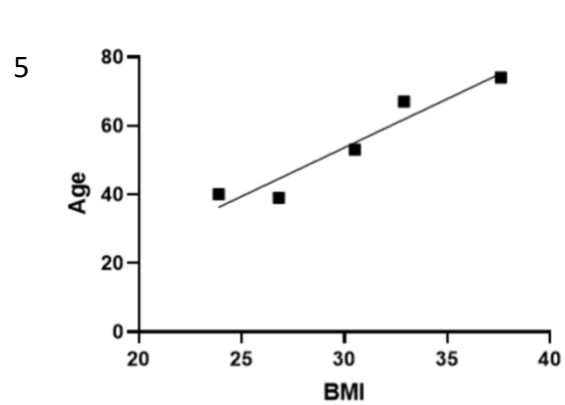


Figure 5: shows the linear regression between age and BMI. $R^2 = 0.9228$ and $P = 0.0093$.

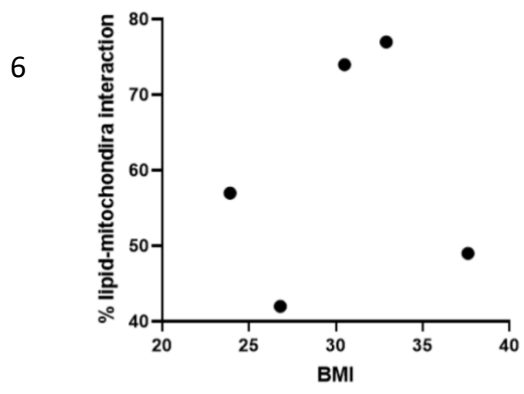


Figure 6: shows the multiple linear regression. $R^2 = 0.09666$.

The data suggests that this study would benefit from repetition in a larger sample size. It would also be useful to repeat in a male cohort to analyse for sex-based differences.

1.2 Research Impact

The development of a new staining technique is significant for future scientific research it can allow the study of mitochondrial-lipid interactions in a way that it has not been studied before. This opens the field to a technique that can be used in a range of new clinical and experimental applications. This is valuable for identifying mitochondrial dysfunction in a range of diseases.

This discovery also provides implications for the development of other staining techniques. Mitochondrial tracking stains may have the potential to be used with other staining agents to consider other mitochondrial interactions. Additionally, this provides new insights into live cell stains which could be applied to non-living cells which was not considered possible before. This allows for mitochondrial research to be used in vitro studies as whole tissue samples can be investigated not just monolayer cell culture.

5 patients of an all-female group were selected for this research. In future this could be repeated on a male cohort to also understand sex-related differences between patients. This could also be used as a comparison between medicated and unmedicated patients to develop better treatments for improving metabolic and cardiovascular health.

1.4 Dissemination of Research

I organised the opportunity to present my research poster alongside a short pitch to fellow researchers in LICAMM including PhD students, technical staff, and principal investigators. This improved my confidence in presenting also gave me the opportunity to gain feedback from valuable mentors. I took advice about how to improve the flow of my pitch as well as what parts of my project to emphasise.

As this co-stain is a newly developed technique, I am also aiming to publish an article in the Journal of Lipid Research which showcases new techniques that are available to researchers to study lipids with more detail. Records with the new protocol are available to researchers at LICAMM which can be used in research for years to come.

2. Skill Development

Throughout my time at LICAMM, I have gained valuable scientific skills including cryosectioning and operating complex computer and microscope software. I have also used this time to build upon my core Laidlaw values. During my application, I was tasked with describing which value described me best which at the time felt like a challenge to find examples of these values. Now I have been able to demonstrate these values continuously.

2.1 Laidlaw Values

Ambition is ingrained in academia; you find yourself considering so many methods and techniques all at once. You are constantly picking up ideas from other people and thinking of new ways to further your research. Initially, my project was focused on histology, but by the end of my placement, I had explored mouse research, cell culture, and protein analysis. I now feel inspired to pursue a career in research and I am ambitious to lead projects in the future.

I tested my bravery first by volunteering to take part in mouse dissections. I had never seen a dissection before let alone conducted one myself, so it felt intimidating. Watching the demonstration, I felt scared and guilty, but I persevered and pushed myself to attempt the next dissection. As I began, a fellow placement student had to leave as she felt uneasy which meant my supervisor had to temporarily leave me alone for the dissection. At first, I gave up on trying to collect the first organ and waited for her to return and guide me, but as I waited, I realised the importance of taking initiative and dove into it. When my supervisor returned, I had collected all but one of the samples and was ready for a second go. I felt extremely proud of myself for pushing through my nervousness.

Curiosity was the trait I used to describe myself in my application due to my love for scientific discovery. I continued to develop this quality throughout my placement as I constantly engaged with or asked questions about the other research that people were doing at LICAMM.

A core part of determinism is resilience, to push past setbacks and keep going. I found this to be the quality most tested while at LICAMM. In the fourth week of my placement, I was met with a brick wall. After 2 weeks of preparing and refining my

technique on the cryostat, I was ready to start cutting my patient samples. As I went to turn on the machine, I noticed it was not working and after consulting my supervisor we realised it may not be usable for the rest of my time. This was a major setback for me as my project relied on these samples, and I felt worried that I would not have anything to work with. I stayed determined and began to adapt; I started to focus my attention more on the staining technique whilst contacting others who may have access to a working cryostat in the University of Leeds. Eventually, this setback ended up helping push me forward as it pushed me in new directions allowing me to focus on refining the co-stain and it also was the reason I got to work with live cells. Everything about science is extraordinary. Cryosectioning is a very delicate technique where small samples of frozen tissue are cut in a cold chamber into tiny slices. For reference, my samples were around 1cm big and cut to 10µm is 0.001cm thick and must be handled with paintbrushes. I faced constant issues while refining this technique, but I continued to troubleshoot and ask for help from others. This involved asking Dr. Fiona Bartoli (who had lots of experience working with the cryostat) to come in and watch my technique to further improve and perfect it. She also helped me confirm damage in samples and ways we could minimise it in the future. In the end, it took me around 2-3 weeks to complete cryosectioning which involved many long days sitting at the cryostat for hours with freezing hands. At first, I did not think this was extra-ordinary, but I realised I was patient, persistent and used every resource I could to perfect my technique which by the end was extraordinary.

At first, 6 weeks seemed like forever and more than plenty of time to get everything done. But by my second week, I had realised just how many things I wanted to do and see so I realised the need to be fast. I started organising time with various researchers including working on mice dissection and oroboros with Aysha Ali and shadowing Hannah Smith as she worked on cell culture. I also organised a meeting with Professor Ian Roberts making sure to take every opportunity that I could.

I had the greatest opportunity to build being good when engaging in the first residential. All of the sessions and even the free time worked on mine and the other

scholars' ability to connect and collaborate. From working on a short presentation with people that I had only met the day before, to having great fun in the quiz after our dinner. Everyone is so welcoming in the Laidlaw community, and it truly does foster the kindness within yourself, I have built so many meaningful relationships so far and there are many more to come.

2.2 Leadership Development

When I first started at LICAMM, I struggled to imagine how I would build upon my ongoing leadership skills as I lacked the education and training to be able to 'lead' anything. However, I soon learnt how opportunities to build on this do not necessarily require me to lead someone else or a certain task. Instead, I was able to learn about leadership from the people who were supporting me. Various people in my supervisor's lab group were welcoming to guided me through and involved me in new processes. Through this, I have learnt that a good leader makes opportunities for their team and allowing them to progress will only strengthen the group. These leaders guided me through involvement allowing me to get stuck into the research to encourage my engagement and learning.

I sat in on a laboratory meeting where the members of the lab group presented a catch-up on their progress to the lab leader Professor Lee Roberts. I found it insightful to watch him lead the meeting and engage with everyone. After the meeting, I set up a 1-1 meeting with him to discuss his career and experience with leadership. We discussed the roles he takes on, the benefits of both academia and industry and most valuable to me, his leadership skills. The key things I took away from the discussion included leading by example, building on your own expertise by utilising other people's abilities and the value of succession planning. We also discussed the approach to conflict resolution using awareness and negotiation to be aware of the ways solutions must arise but also to have the willingness to compromise.

4. Future Plans

This entire experience has been extremely valuable to me, when I first decided to apply for the Laidlaw programme I felt as if I was not enough to be considered, even once I had secured an interview and acceptance onto the programme. Once I

attended the first residential however, I realised I wasn't alone in feeling this way. I spoke to a fellow scholar Lottie Knee, and we discussed imposter syndrome. But by the end of our residential, we had both realised that we were Laidlaw scholars for a reason. We were future leaders and our research was important.

Within the first weeks of my placement, I had been involved in research that I had previously had no understanding of. I engaged in new techniques and learnt from the researchers around me about what a future in research could provide me. This experience provided me with hands-on laboratory experience that went beyond anything I could gain in my undergraduate degree; I have also gained many valuable connections to prepare me for my next steps. I am well-experienced and planning to pursue a year in industry within the scientific industry to further support my ambitions.

Before my placement, I had considered pursuing a career in academia, but I was unaware of what this entailed. Therefore, as I set out to gain this experience, I made sure to take advantage of the range of skills and techniques used here to consider my potential as a researcher. Overall, I have gained a significant amount of experience in various laboratory techniques and protocols that will not only help me throughout my degree but will also support me in securing placements and jobs.

Throughout the project, I have become accustomed to the work at LICAMM, and this has significantly influenced my career plans to which I am now considering pursuing a PhD. I am now confident in applying for a year in industry placement to end my undergraduate degree as a well-rounded individual with a clear career path in mind.

Supervisor

During the research period, Florrie demonstrated adaptability and curiosity by exploring histology, which was a shift from the original project plan. Despite this change in focus and some challenges in scheduling due to my busier-than-anticipated summer, Florrie remained proactive and engaged throughout. She approached the project with enthusiasm, demonstrating resilience when faced with roadblocks and maintaining momentum without letting challenges affect her progress or outlook.

Strengths

- **Problem-Solving:** Florrie consistently demonstrated initiative by attempting to troubleshoot issues independently before seeking guidance, showing both resourcefulness and determination.
- **Independence:** Florrie was confident in taking ownership of her work, balancing self-directed research with knowing when to seek support.
- **Work Ethic:** Florrie accomplished more than expected within the short time frame, producing work of consistently high quality.

Leadership Attributes

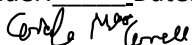
- **Initiative:** Florrie took the lead in exploring new directions for the project, demonstrating intellectual curiosity and self-motivation.
- **Resilience:** She displayed a positive attitude in the face of setbacks and maintained steady progress despite obstacles.
- **Collaboration:** Florrie was unafraid to ask for help when needed, engaging constructively and making the most of mentorship support. She integrated seamlessly into the team, contributing positively to the group dynamic and establishing herself as a valued member of the team.
- **Confidence:** She approached new tasks with assurance and a willingness to try, inspiring trust in her ability to navigate unfamiliar territory.

Areas for Future Development

- **Project Management:** Further developing skills in planning and managing shifting project scopes and timelines will strengthen her ability to anticipate challenges and maintain structure in research.
- **Technical Skills:** Building additional technical expertise in histology methods and related analysis tools could enhance her confidence and expand her research toolkit.

Overall, Florrie has demonstrated excellent potential as both a researcher and a leader. Her independence, resilience, adaptability, strong work ethic, and confidence are standout qualities that will serve her well in future academic and professional endeavours. Continuing to build on these strengths while refining project management and technical skills will support her growth even further.

Signature of Scholar:  Date: 06/09/2025

Signature of Project Leader: _____ Date: _____
 07.09.2025