UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2024/25

NB: This whole report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.

Name of student:

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*(*optional)*

Name of supervisor(s):

Dr J. Arjuna Ratnayaka

Project Title: (no more than 220 characters)

Using SBF-SEM to analyse the choriocapillaris and gain new insights into age-related macular degeneration (AMD)

Project aims: (no more than 700 words)

Age-related macular degeneration (AMD) is the leading causes of irreversible blindness in developed countries(1). AMD affects over 700,000 people in the UK and approximately 150 million individuals worldwide(2). This condition causes the loss of central vision which significantly impacts everyday activities such as reading, driving and recognising faces. End stages of AMD present in two forms, wet and dry AMD. Wet AMD is caused by the abnormal growth of nascent choriocapillary blood vessels of the choroid into the retina. These vessels are leaky so haemorrhage fluid causing retinal detachment and scar tissue formation(1). This type of AMD can be managed with lifelong anti-Vascular Endothelial Growth Factor (VEGF) injections to slow its progression. However, this does not cure AMD(3). Dry AMD is characterised by the presence of subretinal fatty deposits termed drusen and the gradual degeneration of retinal pigment epithelium (RPE) cells, leading to RPE atrophy and subsequent loss of photoreceptors(1). Despite it affecting ~50% of all end-stage AMD patients, there are currently no effective treatments for dry AMD(3). Given the lack of effective therapies for AMD, it is clear that further research is required to unravel its aetiology and disease mechanisms.

This project aims to investigate ultrastructural changes in the choriocapillaris of the choroidal blood supply in the retina of AMD patients compared to non-AMD controls. Human macular (central retina) punch biopsies received from the University of Manchester Eye Repository were used in this project.

Previous studies suggested that there were significant changes occurring in the choroidal vasculature of AMD patients. These included reduced vascular density and the presence of "ghost vessels". The latter term describes vessels that had lost endothelial cells but remained structurally intact, likely without any perfusing blood, in dry AMD patients(4, 5). This data suggests that degeneration of the choriocapillaris may also play a hitherto unknown role in disease progression. While previous studies of this kind have primarily examined donor AMD tissues using two-dimensional histology and transmission electron microscopy, our project aimed to refine these approaches further by employing Serial Block-Face Scanning Electron Microscopy (SBF-SEM). This technique allows for automated, high-resolution collection of serially-sectioned electron microscopy images from resin-embedded tissues to eventually produce a stack of aligned images that can be reconstructed into a three-dimensional dataset. This provides a detailed ultrastructural, three-dimensional view of choroidal and RPE architecture in AMD tissues, a manner not attempted previously(6).

Hypothesis:
We hypothesise that ultrastructural changes in the choriocapillaris are correlated with AMD pathology.

Aim 1: To prepare samples and obtain high-quality SBF-SEM datasets from macular punch biopsies of donor eye tissues from early and intermediate-stage AMD patients alongside age-matched/healthy controls.

Aim 2: To measure features of choriocapillaris vasculature in 2D and 3D, and undertake comparative statistical analyses between early and intermediate AMD samples alongside controls.

Aim 3: To perform 3D reconstruction of the choriocapillaris using software such as Fiji and ITK-SNAP.

By generating three-dimensional ultrastructural data of the choriocapillaris, this study aims to provide new insights into choriocapillaris changes associated with AMD. We anticipate that our data will show possible indications of 'ghost vessel' development through measurements of the endothelial layer, as well as decreased capillary density, consistent with prior findings. Ultimately, this project aims to contribute to a deeper understanding of AMD pathology at the ultrastructural level. The data generated may serve as a foundation for future studies exploring links between choriocapillaris degeneration and disease progression through 3D-reconstructions. In the longer term, such findings could help identify potential treatments to delay or prevent choroidal atrophy in patients with dry AMD.

References:
1. Bhutto I, Lutty G. Understanding age-related macular degeneration (AMD): relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. Mol Aspects Med. 2012;33(4):295-317.
2. Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2(2):e106-16.
3. Khandhadia S, Cherry J, Lotery AJ. Age-related macular degeneration. Adv Exp Med Biol. 2012;724:15-36.
4. Sohn EH, Flamme-Wiese MJ, Whitmore SS, Workalemahu G, Marneros AG, Boese EA, et al. Choriocapillaris Degeneration in Geographic Atrophy. Am J Pathol. 2019;189(7):1473-80.
5. Mullins RF, Johnson MN, Faidley EA, Skeie JM, Huang J. Choriocapillaris vascular dropout related to density of drusen in human eyes with early age-related macular degeneration. Invest Ophthalmol Vis Sci. 2011;52(3):1606-12.
6. Courson JA, Landry PT, Do T, Spehlmann E, Lafontant PJ, Patel N, et al. Serial Block-Face Scanning Electron Microscopy (SBF-SEM) of Biological Tissue Samples. J Vis Exp. 2021(169).

7. Ratnayaka JA, Keeling E. Serial block face scanning electron microscopy reveals novel organizational details of the retinal pigment epithelium. Neural Regen Res. 2022;17(3):569-71.
8. Leighton SB. SEM images of block faces, cut by a miniature microtome within the SEM - a technical note. Scan Electron Microsc. 1981(Pt 2):73-6.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Project Outcomes:

Aim 1: Five samples were prepared for SBF-SEM(7) based on established methodologies(8), including two controls, one early AMD, and two intermediate AMD samples. Following tissue processing, semi-thin resin sections were taken from each sample using ultra-microtomy to identify and isolate 1mm^3 regions of interest to be mounted on pins for SBF-SEM. During SBF-SEM imaging, each tissue block on the pin was repeatedly sectioned into ultrathin slices by a diamond knife inside the microscope's chamber. After each section, the block face was imaged, producing a sequential stack of high-resolution micrographs for each sample. The resulting image stacks were processed and analysed using Fiji (Figure 1).

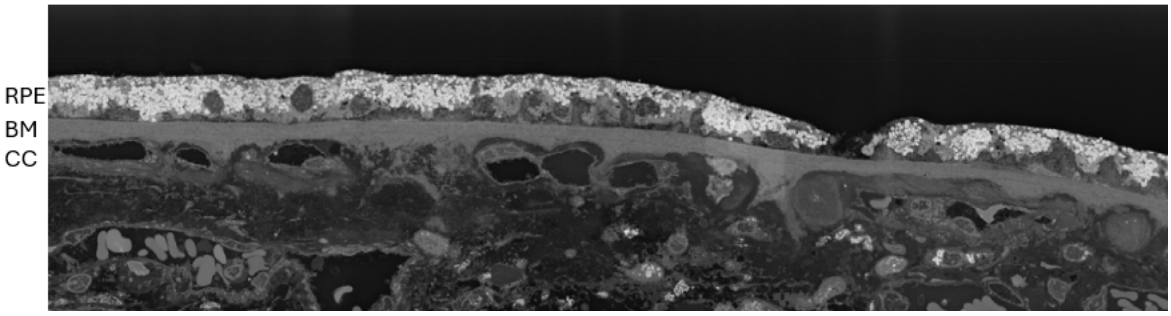

Figure 1 *An example of a stitched tiles SBF-SEM slice from a stack of images for an early/intermediate AMD patient showing the choriocapillaris (CC), Bruch's membrane (BM) and retinal pigment epithelial (RPE) layers.*

Aim 2: Once processed, the image stacks were used for two and three-dimensional measurements. By systematically examining the image series, we developed a schematic representation of vascular anatomy termed a "2.5D tree" (Figure 2). This visualisation provides a two-dimensional depiction of the three-dimensional course of each vessel through the image stacks, illustrating where vessels start, end, merge or branch. This approach enabled us to identify measurement points and to visually compare the choriocapillaris networks between healthy controls vs. AMD samples.

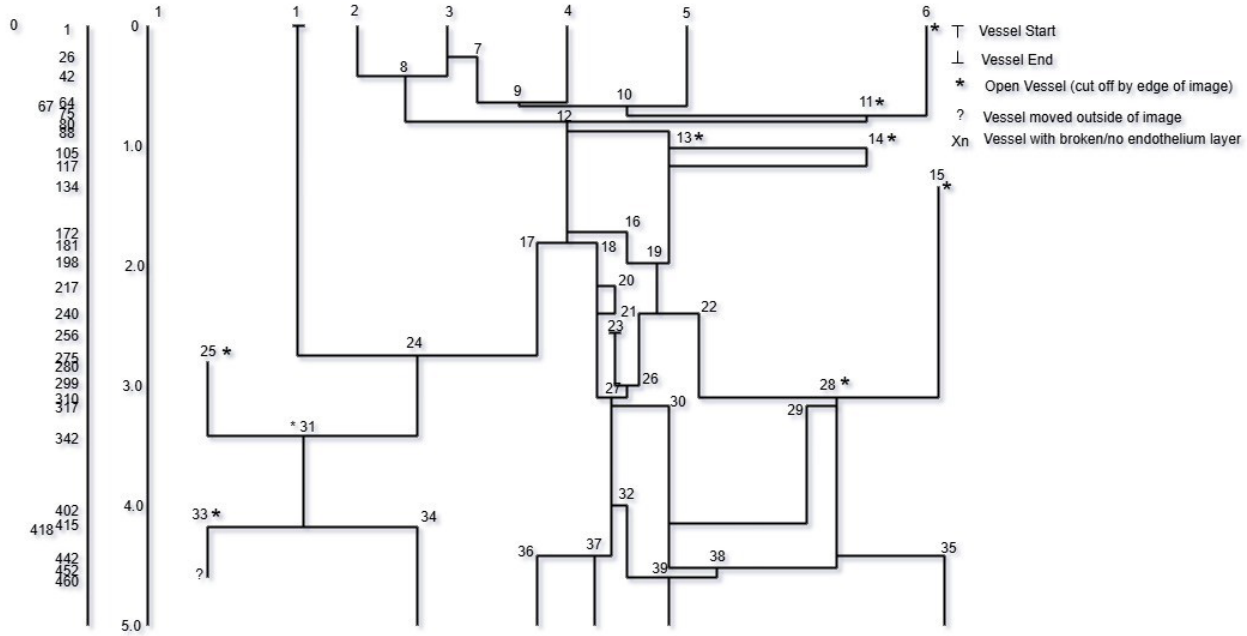

Figure 2 *Example of a 2.5D tree diagram of an Early AMD patient.*

Using Fiji, three measurements (start, middle and end) were taken for each vessel within the stack. The polygon tool was used to delineate around the vessels and lumen area, allowing estimation of the vessel/lumen diameter, circularity and average endothelial layer thickness (Figure 3).

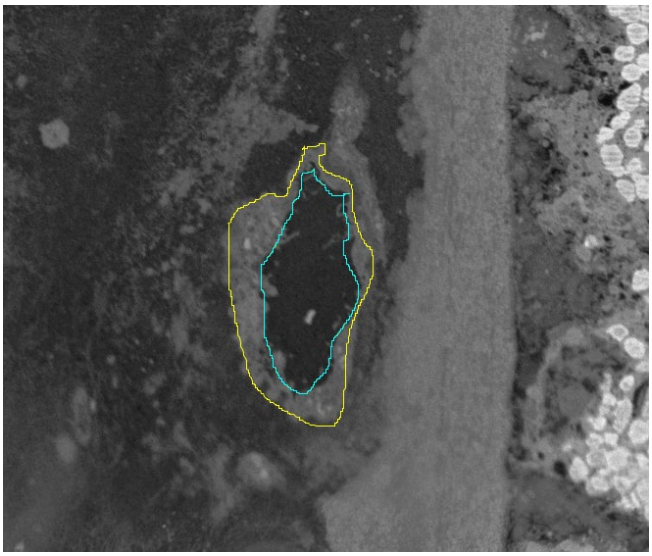

Figure 3 *Example of usage of the polygon tool on Image J to measure thickness of Endothelial layer.*

Results and Discussion:

From the 2.5D trees we were able to identify a pattern with branching (the number of times vessels merged/separated) in our datasets. While no significant differences were observed between control and early AMD samples, there was a notable decrease in branching frequency from control and early AMD to intermediate AMD samples. Within a 5µm depth of the choriocapillaris, vessel number decreased from 51 (control) to 39 (early AMD) and 15 (intermediate AMD).

Diminished branching likely reflects a decrease in vascular density as AMD progresses, consistent with prior findings suggesting the likelihood of increased 'ghost vessel' development as more blood vessels become atrophic, concomitant with subretinal drusen formation(4, 5). A caveat to this interpretation is that we were unable to definitively identify 'ghost vessels' with SBF-SEM. Furthermore, our samples did not show the RPE layer continuously through our dataset, so we were unable to correlate any loss of vasculature to atrophic RPE or drusen through our own findings.
Furthermore, measurements of the endothelial layer thickness did not reveal any correlation with AMD progression. While one intermediate AMD sample showed slightly reduced endothelial thickness (averaged thickness of 12.63 µm) compared to control (14.79 µm) and early AMD (16.37 µm), significant variability across samples excluded any indication of broader links. A larger sample size would therefore be required in future studies to gain greater statistical power for analyses of this kind. However, a possible development is that quantification of averaged endothelial thickness may be unsuitable for assessing choroidal atrophy, warranting adoption of more refined approaches to detect localised changes within smaller regions of choroidal vessels. In addition to endothelial thickness, I also recorded other parameters such as vessel diameter, area, perimeter and vessel circularity. Analysis of these is ongoing and will be presented at the Society Summer meeting.

Experience Gained: Through this project I was able to strengthen my ultramicrotomy skills and gain valuable hands-on experience in SBF-SEM sample preparation, imaging and analysis. I also developed a solid foundation in image processing, 3D reconstruction and quantitative analysis using Fiji, which has enhanced both my technical competence and confidence in handling large microscopy datasets. Over the ten weeks, I learned extensively about the structure of the eye and AMD through literature reviews and weekly lab and group meetings. Working closely with other researchers also gave me valuable experience in collaboration, communication and problem-solving within a research team environment.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Summer 2026

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

I will submit a poster or an oral presentation at the AS Summer Meeting 2026

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Using SBF-SEM to analyse the choriocapillaris and gain new insights into age-related macular degeneration (AMD)
We used 2D and 3D microscopy methods to find out if there were any changes to blood vessels under the retina that is linked with developing central vision loss. Damage to tissues in the central retina leads to a common, irreversible blinding condition called age-related macular degeneration (AMD) which has no effective treatments. AMD affects 1/3 individuals over the age of 70 years and approximately 700,000 people in the UK. For a long time, damage and eventual death of tissues in the central retina (called the

macula) were understood to play a key role in AMD. However, recent evidence shows that damage to the blood vessels that supplies oxygen and nutrients to these tissues in the macula, also play an important but poorly understood role in developing AMD. To study this further, we used an altogether new approach called serial block face-scanning electron microscopy (SBF-SEM) that provides information in three-dimension. This technique offers an exciting way to study changes to the retinal blood supply (called the choroid) that may provide new ways of understanding this complex disease. We hope our discoveries will pave the way for developing new ways of treating AMD patients in the future.

Other comments: (no more than 300 words)

Modifications were made to the original project plan, which initially proposed carrying out a full 3D reconstruction of the choriocapillaris using Fiji and Amira software. After testing semi-automated reconstruction methods during the first week, it became clear that a complete 3D reconstruction was not achievable within the project timeframe. Instead, we adapted the approach to focus on constructing "2.5D trees" from the SBF-SEM image stacks and performing quantitative vascular measurements, which provided meaningful structural insights. The datasets developed here will serve as a foundation for future research and the project may be taken up by another student in the future to complete the 3D reconstruction component.

Data Protection/GDPR: I consent to the data included in this submission being collected, processed and stored by the Anatomical Society. Answer YES or NO in the Box below
YES
Graphical Images: If you include graphical images you must obtain consent from people appearing in any photos and confirm that you have consent. A consent statement from you must accompany each report if relevant. A short narrative should accompany the image. Answer N/A not applicable, YES or NO in the box below
N/A
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*Signature of student …*Srishti Ramrakhiani*……. Date…15/10/2025……..*

Signature of supervisor……Arjuna Ratnayaka Date…19th October 2025……..

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