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Measurement of follicle density and diameter

THE UNIVERSITY of ADELAIDE MINIPROBES

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Executive Summary

For wool producers, sale price of wool is largely determined by quantity and quality (primarily diameter) of wool fibres. Decreasing the diameter of the wool by one micron will increase its value by approx. 10%. However, selecting sheep with high quantity (high follicle density) and quality (small diameter) wool is difficult. There is currently no practical way to measure the wool follicle density. We have developed a new type of optical scanner that can identify the wool follicles and shafts in a fresh skin biopsy sample within a few minutes of the biopsy being taken from the sheep, avoiding the cost and delays of histology. In this project, we established the feasibility of this technology and showed that it was also possible to automate follicle density estimation using an artificial neural network algorithm. We recommend developing this technology into a commercial product for the Australian wool industry.

In parallel, we also developed a technique to image the tiny blood vessels in the sheep's skin using in vivo optical imaging. These blood vessels are critical for heat / cold tolerance of the sheep. Using this new technique, we show the first ever images that visualise the skin blood vessels in a sheep. This technique has potential to help wool researchers and physiologists understand how to select for sheep with a robust ability to regulate their body temperature.

Contents

1. Introduction/hypothesis

For wool producers, 70%-80% of the sale price of wool is determined by quantity and quality (primarily diameter) of wool fibres [1, 2]. Decreasing the diameter of the wool by one micron will increase its value by approx. 10%.

Quantity (clean fleece weight) and quality (fibre diameter) are both phenotypically and genetically unfavourably correlated [3]. However, by selecting sheep with greater density of wool fibres we can improve both quantity and quality. In addition, wool follicle density is highly heritable [4] so genetic gains can be made if the trait can be measured cost effectively.

At present, to measure wool follicle density, a producer must use histology, which involves a small biopsy sample of the animal's skin, chemically processing it and analysing it under a microscope. This process costs >$100 per animal and can take several weeks. Despite the value of the measurement, few farmers find this process to be either economically or logistically viable.

Thus, if they were able to more readily identify high value sheep at an early stage, they could significantly increase the rate of genetic improvement and profit in the flock. In parallel, by identifying sheep with low quality, coarse wool at a young age, farmers can eliminate these sheep from the flock at an early stage.

There is no product available on the market that can assess the potential wool production of a lamb prior to their first shearing.

In this project, we are exploring a new imaging technology to measure the wool follicles that produce the wool. These follicles are located just below the surface of the skin. The technology we use is called optical coherence tomography (OCT). It is a medical imaging technology that is commonly used in the ophthalmology and cardiology departments of many hospitals. We have re-purposed this technology for use in livestock. Our innovation is in customising the scanners for use on the farm, and in the development of intelligent machine learning software to automatically quantify the wool follicles. Scans will be performed on fresh skin biopsy samples immediately after they are removed from the sheep, providing 'instant histology' and avoiding the need for histological processing and sectioning.

In addition, OCT is also able to image the small blood vessels in the skin. This microvasculature is important both in helping sheep to regulate their body temperature and in supporting wool growth. We have previously used this technique to image these skin blood vessels in humans. However, it has not been tested with sheep. In the second part of this project, we will assess the feasibility of using OCT imaging to visualise the microvasculature in sheep skin.

2. Literature Review

Optical coherence tomography (OCT) [5] is analogous to ultrasound imaging. However, while ultrasound acquires scans of tissue by measuring the reflections of sound waves, optical coherence tomography uses near infrared light. It is commonly used in human medicine, specifically in ophthalmology [6] and cardiology [7]. OCT is able to acquire much higher resolution images than ultrasound, with a typical resolution of 3-15µm. However, it is not able to image deep below the skin surface, being limited to an imaging depth of 1-2mm. This makes is well suited to imaging the microarchitecture of the skin, with the potential to visualise wool shaft and follicles.

OCT is also able to image blood flow. To achieve this, we use image processing algorithms to analyse the scans for traces of blood movement. An OCT image contains speckle noise. This is a random pattern of dark and bright pixel intensities that overlay the OCT image. Speckle is time-invariant. That is, if we were to image some tissue and the OCT scanner and the tissue did not move, then the noise pattern of dark and bright pixels will remain constant and unchanging. When we image tissue containing a blood vessel, then the constant movement of the red blood cells causes the speckle pattern to change at the location of the blood vessel. By analysing the statistics of how the speckle changes in an image, it is possible to automatically identify the location of blood vessels. In previous research, our team have used this technique to analyse skin blood vessels in humans for a range of applications, including assessing burns scars and diabetes [8-10]. In this project, we are adapting these techniques to image skin blood flow in sheep.

3. Project Objectives

This project has two key objectives:

Objective 1: Establish technical feasibility of acquiring OCT scans on sheep to visualise and quantify wool follicle density.

Objective 2: Establish technical feasibility of OCT to assess the microvasculature of sheep skin in vivo.

4. Success in Achieving Objectives

Both objectives were successfully achieved.

Objective 1:
We developed a protocol to visualise the wool follicles and shafts using OCT. We explored two approaches: in vivo imaging of the skin on the sheep; and ex vivo imaging of a fresh skin biopsy sample taken from the sheep.

In vivo imaging is desirable as it avoids taking a biopsy sample. However, it is also significantly more difficult and error-prone. OCT imaging is very high resolution and even micron-scale movement while scanning will blur the images. This slows the rate at which we can scan sheep as the scanhead must be carefully positioned on the sheep's skin and then the operator must wait for the sheep to stop moving. It would be difficult to do this type of scanning at the speed required for a commercial operation.

To increase the rate at which we could scan sheep and also reduce movement artifact in the images, our team developed a technique to reliably image an 8mm skin biopsy sample taken from the sheep. By scanning both sides of the skin sample, we were able to image the wool shafts (outer side) and the wool follicles (inner side).

Objective 2:
Our team developed a technique to image the skin microvasculature of the sheep. Unlike scans for follicle density, blood flow imaging must be performed in vivo (because ex vivo samples have no blood flow). Our team developed a custom fitting to allow the OCT scanhead to be positioned on the sheeps' skin. Whilst these scans were slower than the wool follicle scans, we were able to successfully acquire these scans on a range of Merino and crossbreed sheep.

5. Methodology

a. Scanning protocol for wool follicles

All experiments were approved by the University of Adelaide Animal Ethics Committee (approval no. S-2020-002). We scanned 30 sheep, being a mixture of Merino and Border Leicester x Merino. The sheep were approximately 4 months old.

Prior to imaging, wool was clipped from a small section of the sheep's skin. Wool interferes with the light beam used to acquire OCT scans, and so needs to be removed to avoid shadowing artifacts in the images. An 8mm punch biopsy of the skin was then taken. The inner surface of the biopsy sample was often covered by a thin layer of highly opaque fascia which was manually removed with a scalpel. The biopsy sample was then placed in a custom tissue holder designed and 3D printed for this project to enable optimal imaging. OCT scans were acquired on both sides of the sample. The outer side showed the wool shafts, while the inner side showed the wool follicles.

OCT imaging was performed using a commercial imaging system (Telesto III, Thorlabs GmbH, Germany) with central wavelength of 1300nm and an axial resolution of 5µm in tissues (assuming refractive index of 1.43 for the skin) [11].

Scanning was performed using a detachable imaging probe (OCT-LK2, Thorlabs, GmbH, Germany) with a lateral resolution of 7µm. Scans were visualised using software developed in Matlab (Mathworks, USA).
The scanning protocol is illustrated in Figure 1, showing clipping of the wool, acquiring a punch biopsy and optical scanning.

Figure 1: (left) clipping wool close to the skin. (centre) obtaining an 8mm biopsy. (right) Optical scanning of biopsy sample.

b. Automated follicle density estimation

There are several different aspects of the OCT scans that could be automatically quantified. These include counting the number of follicles; counting the number of wool shafts; calculating the ratio of follicles to shafts as an indicator of the secondary-derived follicles; or computing the average wool shaft diameter. Within the scope of this initial project, we developed software to automatically count the number of follicles in each scan. Our goal was to demonstrate that automated computer algorithms can be successfully applied to analyse OCT scans of sheep skin. Future projects could extend this to quantify other aspects of the skin.

Using deep learning algorithms, there are several different ways to tackle this problem. We have chosen to use an approach that performs a categorisation task instead of a pixel-by-pixel segmentation.

Rather than exhaustively counting every follicle, the algorithm separates the scan into small subimages, estimates the number of follicles in each and then adds these together. This is conceptually similar to the way that a person may glance at a picture and roughly estimate the number of things in it. Our algorithm examined each small subimage and categorised it as containing either <=2 follicles, 3-4 follicles; 5-6 follicles, >=7 follicles.

Our algorithm uses a type of convolutional neural network referred to as ResNet50 [12]. ResNet50 is a variant of the ResNet model which has 48 Convolution layers along with 1 MaxPool and 1 Average Pool layer. The network architecture is shown in Figure 2. Resnet is a feature extraction network. Different layers of the network identify image features such as lines, edges or corners or basic shapes. These are then fed into subsequent layers to extract more complicated features.

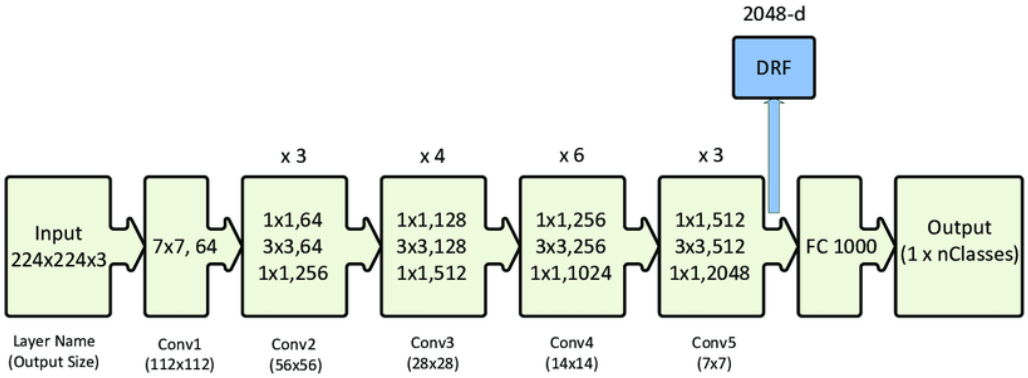


Figure 2. Resnet50 architecture. Key: The notation k × k, n in the convolutional layer block denotes a filter of size k and n channels. FC 1000 denotes the fully connected layer with 1000 neurons. The number on the top of the convolutional layer block represents the repetition of each unit. nClasses represents the number of output classes. Image taken from [13].

The network is pretrained on images taken from ImageNet [14]. This is a publicly available database of several million images, each of which have been categorised by ResNet50 into one of 1000 categories. To adapt the network for counting wool follicles, the 2nd last layer of the network (which is responsible for assigning an image to 1000 categories) was replaced with two distinct layers, allowing two simultaneous outputs from the network.

The first is a 4-output layer that will categorise each small subimage based on how many follicles it contains (<=2 follicles, 3-4 follicles; 5-6 follicles, >=7 follicles). The second is a 2-output layer which will categorise the subimage as either belonging to a Merino or non-Merino breed. In our experiments, the non-Merino sheep were Border Leicester cross Merino. We found that allowing the network to identify the breed of sheep improved accuracy. The network was trained using Adam [15], a momentum-based stochastic optimiser, and used cross entropy as the error metric.

A single image slice at a specific depth below the tissue surface was chosen from each 3D OCT scan, such that it intersected many of the wool follicles. Individual follicles were manually identified within each slice using the image labelling tool, LabelBox (LabelBox Inc., USA). The top half of each image was used for training, and the bottom half for testing. This allowed us to assess the algorithm over all sheep scans.

c. OCT scans of skin blood vessels

In a separate experiment, we developed techniques to acquire OCT of the skin microvasculature. The sheep assessed for these scans were approximately 2 years old. Scans were acquired using a different scanhead (OCT-LK3, Thorlabs, GmbH, Germany). This scanhead has a lower imaging resolution (13µm lateral resolution) but has a longer depth of field which is useful for imaging the blood vessels. A custom 3D printed fitting was developed and attached to the scanhead to allow it to be held on the sheep's skin to minimising movement during scanning. Figure 3 shows the system being used to acquire OCT images.

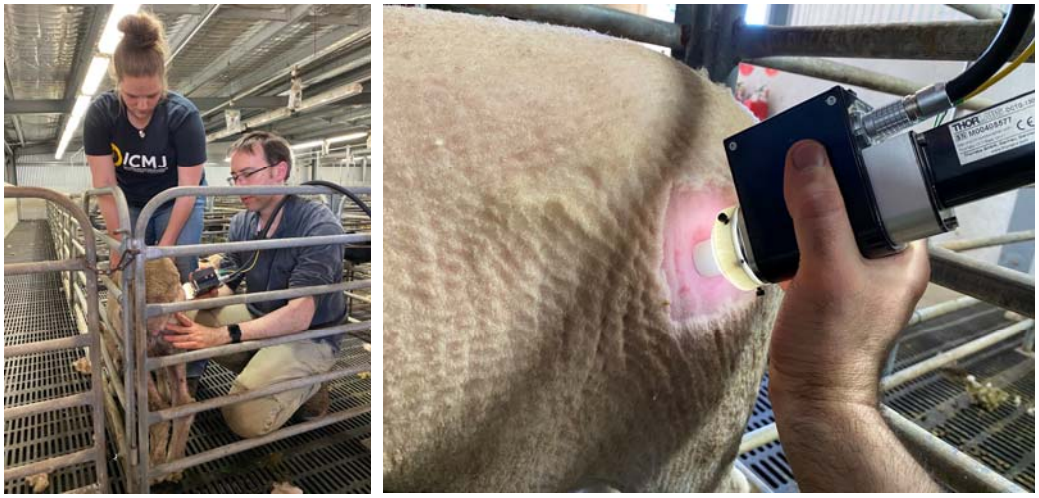


Figure 3: (left) Acquiring OCT scans of the skin blood vessels. (right) Close-up of the scanhead.

6. Results

a. OCT scans of follicles and wool shafts

A total of 30 scans were acquired on a selection of Merino and crossbreeds (Border Leicester cross Merino). The images below show representative scans. Each fresh biopsy sample was scanned twice with the OCT scanner: once on the skin-side which showed the wool shafts; and once on the inner surface which showed the wool follicles. For each image, we also show an H&E histology section taken from the same biopsy sample.

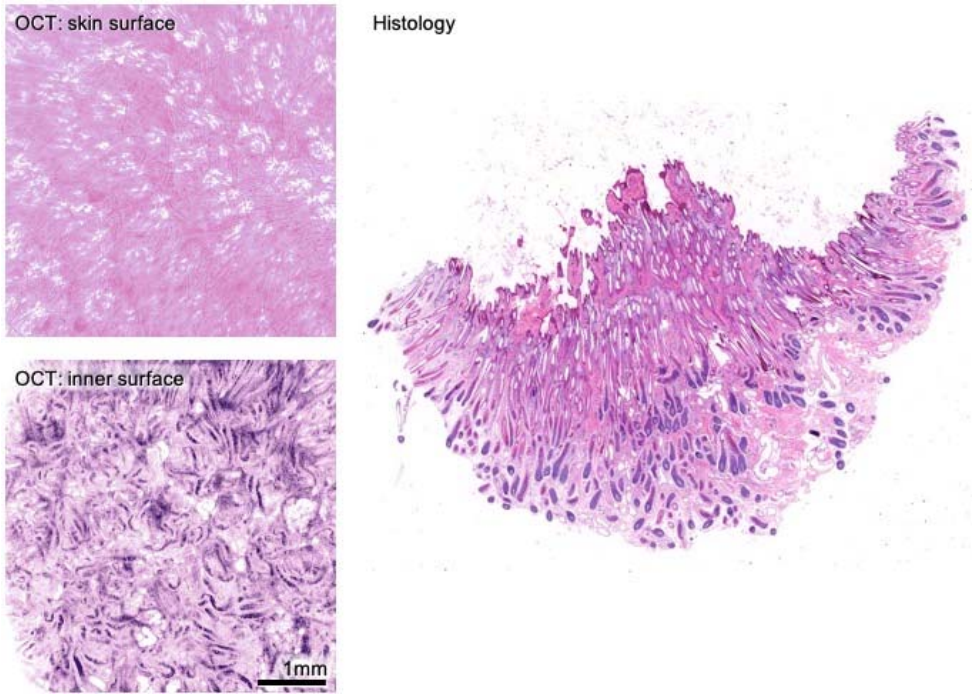


*Figure 3. **Border Leicester cross Merino**. (left, top) OCT scan of fresh biopsy sample taken on skin side showing shafts. (left, bottom) OCT scan taken on inner side of biopsy showing follicles. (right) Histology section taken from the biopsy sample.*

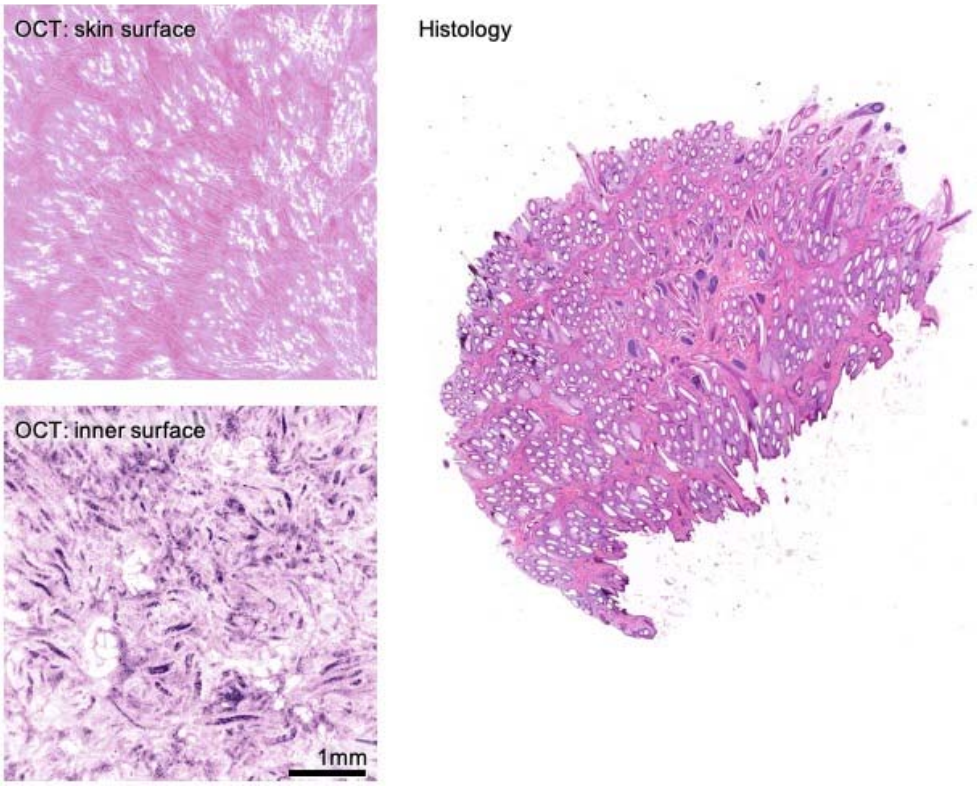


Figure 4. ***Border Leicester cross Merino****. (left, top) OCT scan of fresh biopsy sample taken on skin side showing shafts. (left, bottom) OCT scan taken on inner side of biopsy showing follicles. (right) Histology section taken from the biopsy sample.*

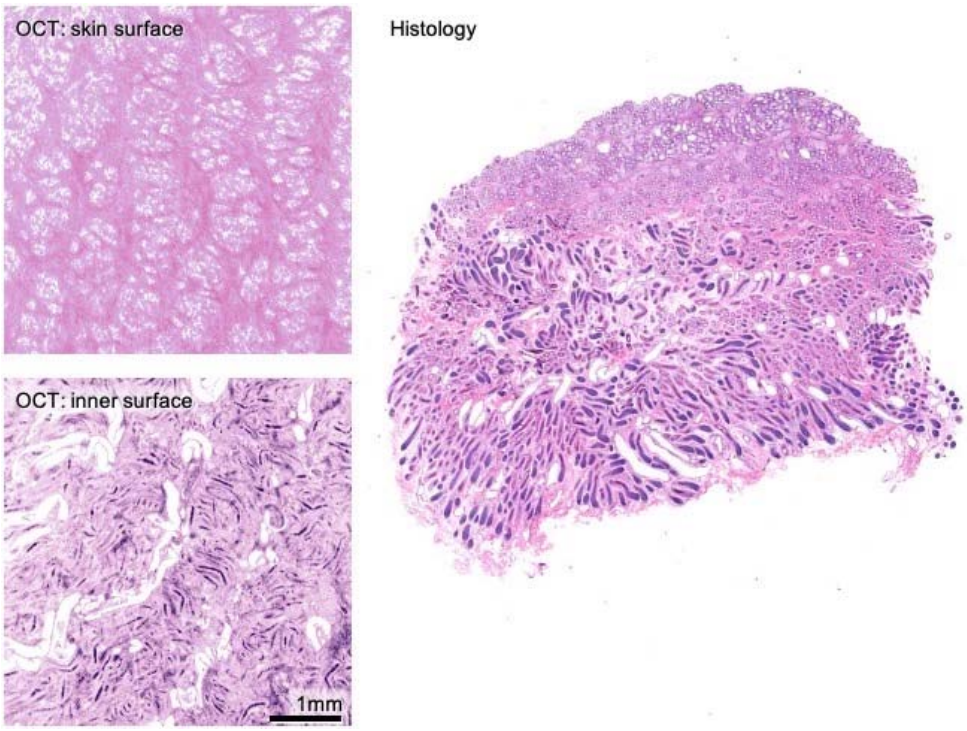


Figure 5. ***Merino****. (left, top) OCT scan of fresh biopsy sample taken on skin side showing shafts. (left, bottom) OCT scan taken on inner side of biopsy showing follicles. (right) Histology section taken from the biopsy sample.*

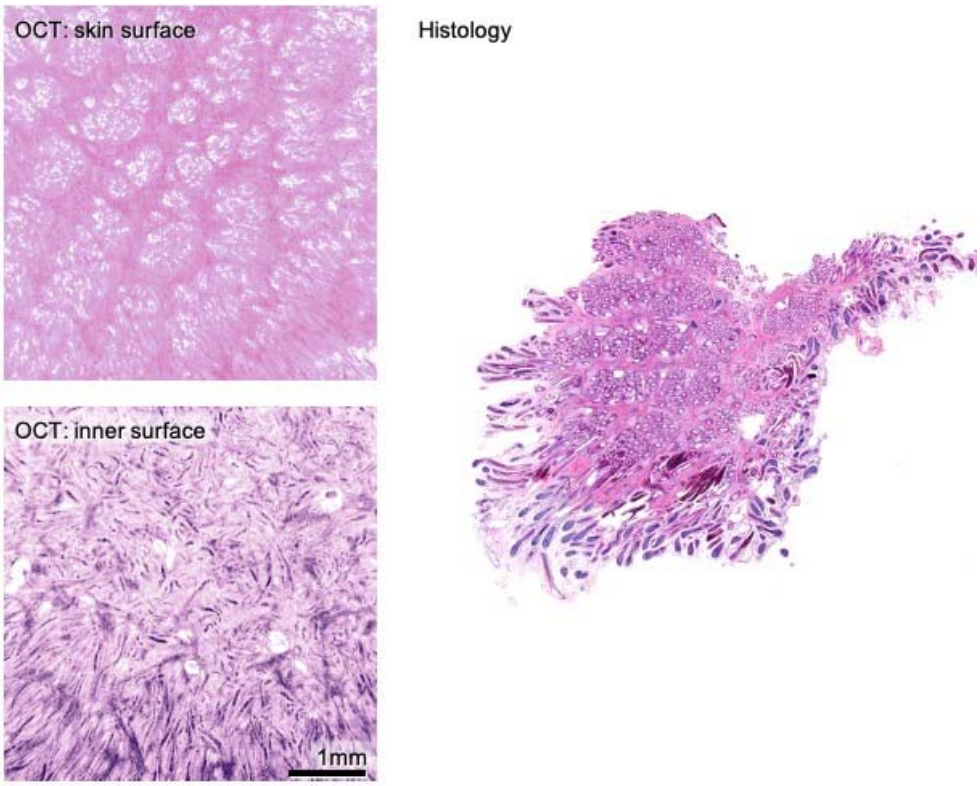


*Figure 6. **Merino**. (left, top) OCT scan of fresh biopsy sample taken on skin side showing shafts. (left, bottom) OCT scan taken on inner side of biopsy showing follicles. (right) Histology section taken from the biopsy sample.*

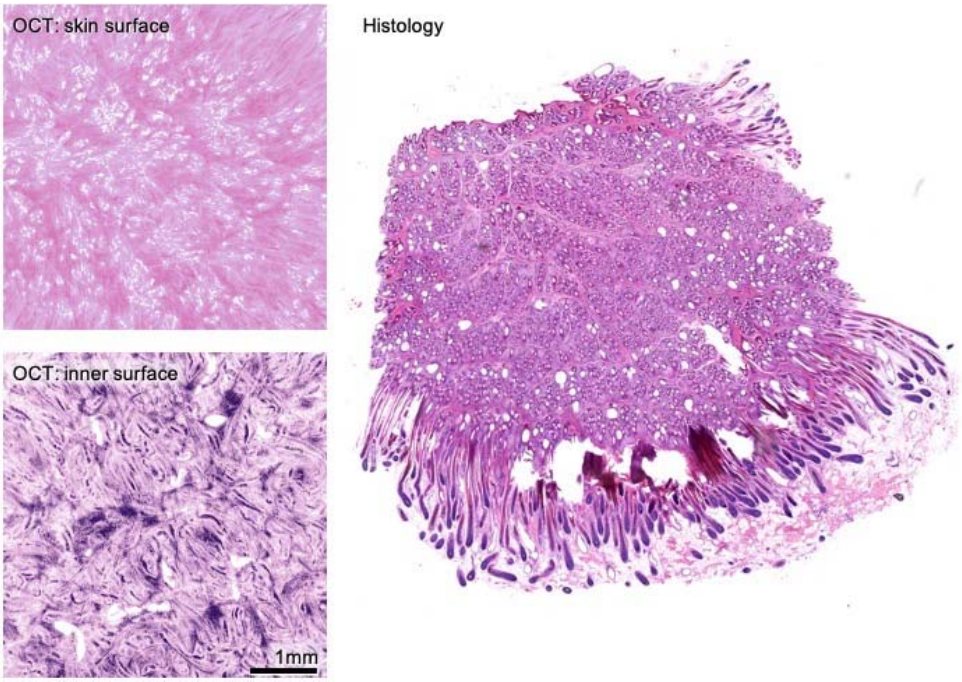


*Figure 7. **Merino**. (left, top) OCT scan of fresh biopsy sample taken on skin side showing shafts. (left, bottom) OCT scan taken on inner side of biopsy showing follicles. (right) Histology section taken from the biopsy sample.*

b. Automated follicle density estimation

The results of automated follicle counting are shown in Figure 8, plotted against a gold-standard value in which the follicles were manually counted. The neural network was able to estimate the number of follicles with an average error of 18%, and an R^2 of 0.8454.

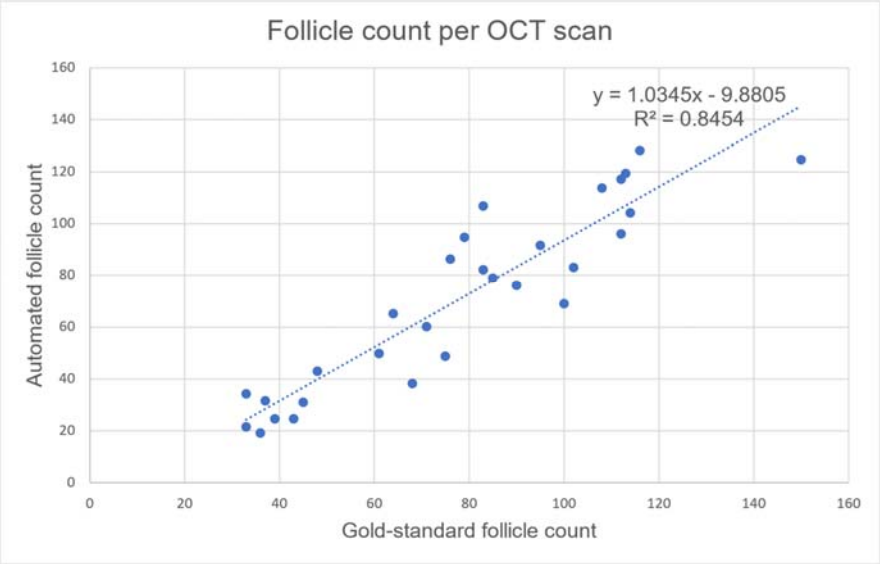
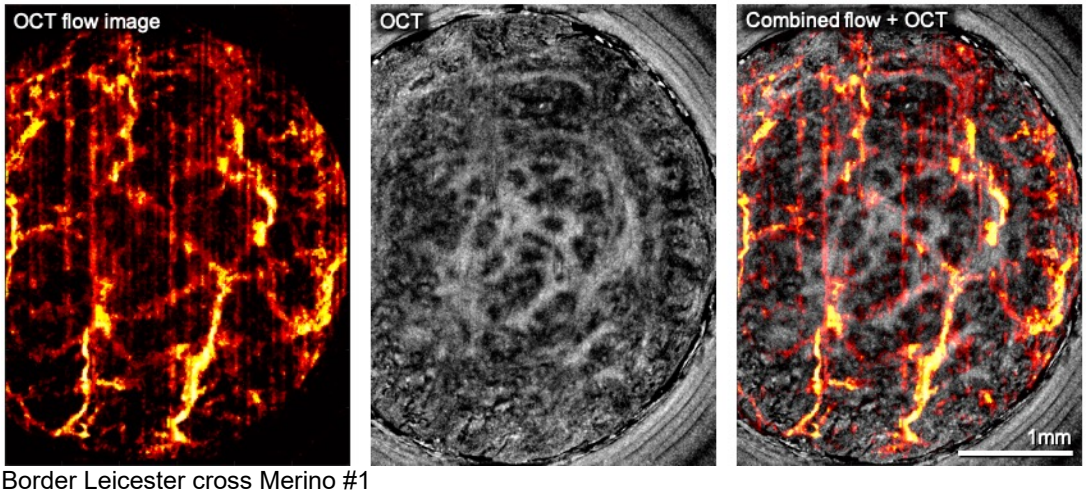


Figure 8. Automated follicle count vs gold standard manual estimates. Each data point corresponds to one 5mm x 5mm OCT scan.

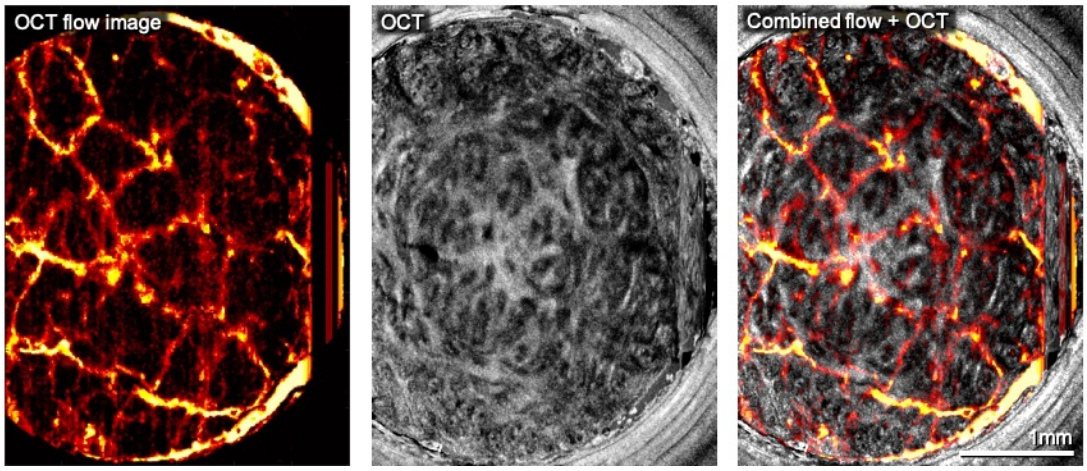
c. OCT scans of skin blood flow

OCT blood flow scans included a selection of Merino and Border Leicester cross Merino. Representative scans are shown in Figure 9 (3x Border Leicester cross Merino) and Figure 10 (3x Merino).

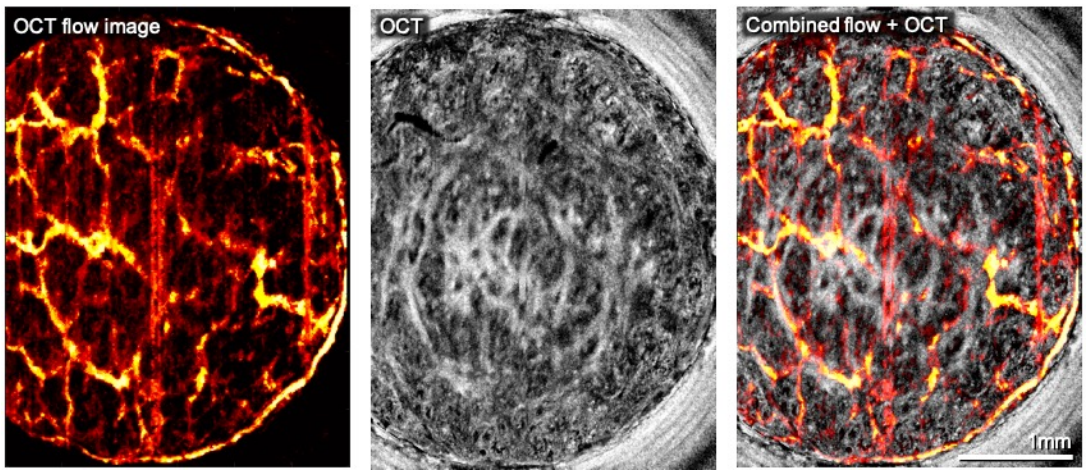
The field of view for the scans is 3mm x 4mm. For each sheep, the left image shows blood vessels coloured in red and orange. Vessels in these images typically have a diameter in the range 30µm - 200µm. The middle image shows a slice taken from the raw 3D OCT scan showing the tissue structure. Note that these images look different to the wool follicle images shown earlier in Figures 3 - 7. This is because the scans are lower resolution hence do not show individual follicles; and have also been coloured in grey instead of histology colouring. However, the collagen bands that delineate groups of wool shafts can be seen. The right image shows the flow image superimposed on the OCT tissue image.



Border Leicester cross Merino #1



Border Leicester cross Merino #2



Border Leicester cross Merino #3

Figure 9. Scans of 3x Border Leicester cross Merino. (left) OCT blood flow image. (middle) OCT tissue scan. (right) Blood flow overlaid on OCT tissue scan.

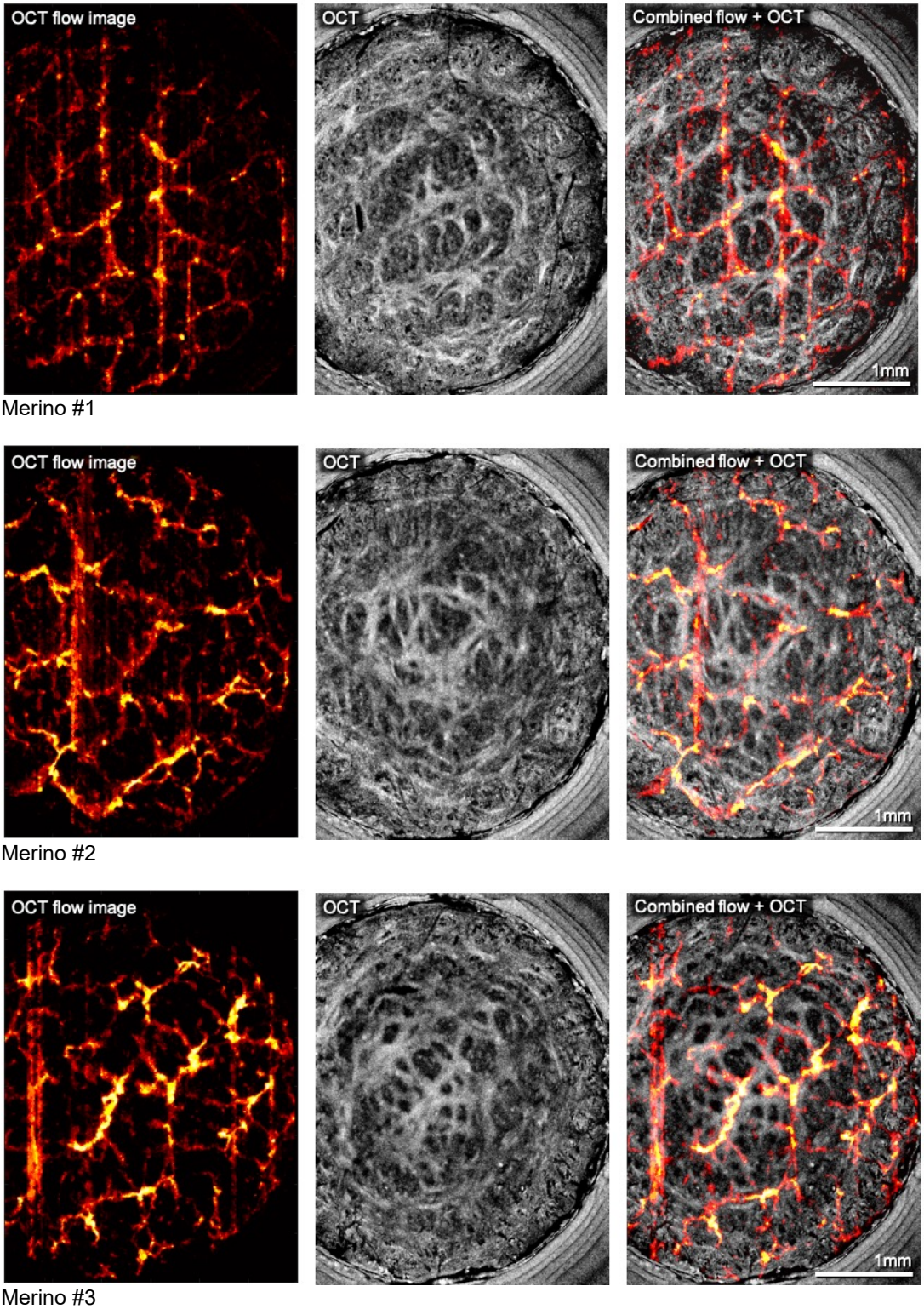


Figure 10. Scans of 3x Merino. (left) OCT blood flow image. (middle) OCT tissue scan. (right) Blood flow overlaid on OCT tissue scan.

7. Commercial Potential

Optical imaging creates new commercial opportunities for the wool industry. The most profitable opportunity is to provide a rapid, low-cost technology to assess wool follicle density. We have identified two potential markets for this device: Stud breeders and Wool producers.

Stud breeders: Stud breeders sell high value sheep to wool producers. Individual sheep can be priced $600 - $60,000 and maximising genetic improvement in their sheep is a critical product differentiator for these breeders. This is high value market and stud breeders are likely to be early adopters and willing to pay a premium for this service. Based on our interviews, we believe that stud breeders would pay $20 per scan per sheep. As an estimate of the market size, the Australian Association of Stud Merino Breeders has over 900 stud breeders registered across Australia, with many other breeds (Dohne, Samm and Border Leister) also seeing merit in this technology.

To estimate a realistic range for the potential revenue from this market, we assume there are 500 - 1000 stud breeders in Australia, each scanning 100 - 400 sheep per year at a cost of $20 per sheep. This would provide revenue for this product in the range $1mil - $8mil per year.

Wool producers: In Australia, there are 13.7mil new Merino lambs per year in wool production, across 12,800 businesses [16]. Our goal is to scan each Merino once, early in its lifetime. Wool producers will see less value in this device than stud breeders, and so would only support a lower cost for the scan. Assuming a price of $5 per sheep, this would give a maximum potential revenue in Australia of $68.5mil per year. A 10% market penetration would yield $6.8mil revenue per year.

Competition: The key competing product to analyse the wool follicles is histology, which costs >$100 per animal. This involves taking a punch biopsy of skin from the sheep and sending the tissue to a pathology lab for analysis. In practice, the process typically takes several weeks, although it could be optimised to a few days. It is not feasible to perform rapid histology on the wool follicles because of the complicated chemical processes required. In contrast, our optical scanner does not require chemical processing of the tissue and hence can be done within a few minutes. With automated analysis software to measure the wool follicles, the entire process could be completed within five minutes.

8. Impact on Wool Industry – Now & in 5 years' time

The potential impact on the Wool Industry will be an improvement in selection accuracy (for breeders) and additional income (for breeders and producers).

This project has established feasibility of using OCT scanning to visualise both wool follicles and wool shafts in fresh biopsy samples. Whilst this still requires that we take a skin biopsy, it avoids the delays and cost involved in performing histological processing. For stud breeders, this technology will provide an important new tool to improve genetic selection of sheep with increased wool follicle density. By demonstrating the feasibility of identifying both follicles and wool shafts, this technology also has the potential to provide an estimate of the number of secondary-derived follicles, where multiple shafts derive from a single follicle.

During this project, we have discussed the technology with a number of stud breeders and service providers for the wool industry. There was a very keen interest in the technology. Based on these conversations, we believe that stud breeders would be prepared to pay $10 - $20 per sheep and that service providers may pay up to $50,000 for a scanner, provided they are convinced that there is sufficient market demand for the service. As a comparison, a pregnancy ultrasound scanner typically costs $16,000 - $25,000, and a wool testing unit costs $80,000 - $100,000.

9. Conclusions and Recommendations

Providing stud breeders with objective measurement tools to improve wool follicle density is critical to enabling the Australian wool industry to improve the value of plain bodied sheep. As the industry reduces the use of mulesing, these types on technologies are critical for the industry to remain profitable.

This project has established the feasibility of OCT to visualise and automatically measure wool follicles. Whilst OCT is a mature technology in the field of medicine, this project is its first use in the wool industry and it will require significant research and development to develop a scanning tool that can be used in commercial stud breeding. We recommend that the wool industry support a follow-on project that is focused on developing a prototype system for industry use. There are several Federal Government funding schemes that may be leveraged to support such a project, such as the CRC-P program and ARC Linkage projects. These schemes require industry investment from non-Commonwealth funds and will then provide additional leveraged funding to support Australian Industry.

The second aspect of this project has been to demonstrate the feasibility of using OCT to visualise the tiny blood vessels in the skin. This technique has potential to provide a powerful new tool to help understand heat and cold tolerance in lambs (thermoregulation) and also to explore how these blood vessels encourage wool growth by supporting good follicle health. The opportunity here is to develop an optical scanning tool that can be utilised by researchers working to better understand these aspects of sheep physiology. We recommend that the wool industry support a research project that will partner engineers with wool researchers to develop this tool and undertake field trials to validate these techniques as an indicator of capacity of sheep to thermoregulate.

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