

final report

Project Code:

Prepared by:

PRTEC.040

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Date published:

PUBLISHED BY Meat and Livestock Australia Limited Locked Bag 991 NORTH SYDNEY NSW 2059

Sensing for Interface Detection and Separation

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

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EXECUTIVE SUMMARY

This report details the work carried out in Milestone 2/3 and is the final report for this project.

An experimental trial was carried out on a beef carcass for the purpose of obtaining samples of hide/connective tissue/subcutaneous tissue from specific areas of the carcass for further testing and analysis and to determine the suitability of the different characteristics of the hide/carcass interface tissue for detection. The types of sensing methods to be applied for detection, and the number and location of samples were decided from the work carried out in milestone one.

The three potential sensing methods trialled were:

- Thermal Imaging
- Low Voltage Radio Spectrum Transmissibility
- Fluorescent Spectroscopy

Two of these sensing methods (Low Voltage Radio Spectrum Transmissibility and Thermal Imaging) were applied to the carcass during the experimental trial and the third method was applied, post trial, to samples taken from the carcass.

Thermal Imaging

Thermal imaging was tested on the beef carcass during the experimental trial and also, later, on carcasses undergoing hide pull at a commercial abattoir processing works. The results of this work show that generally the separation line (hide/carcass interface) is visible in thermograms as a slightly higher temperature zone and becomes more visible (temperature increase) as force is applied to the hide to strip it from the carcass. It is not yet clear why this area shows a higher temperature or what mechanisms might be in operation to cause this effect.

Further research needs to be carried out in this area to determine the nature of these thermal emissions with a particular emphasis on ascertaining any distinctive thermal properties that may exist within the connective tissue at the hide/carcass interface.

If it can be shown that the thermographic method can be developed to consistently and accurately detect the interface then, due to the more mature nature of this technology, there is a greater likelihood that this method would have a practical application for interface detection.

Low Voltage Radio Spectrum Transmissibility

An experiment was set up to determine whether it was possible to accurately discriminate between different tissue types based on the interpretation of a radio signal transmitted through the different tissues. For example, if the connective tissue at the hide interface was shown to be a better transmission medium than the surrounding tissue, then it may be possible to discriminate the two.

The results from the Low Voltage Radio Spectrum Transmissibility method show that, although at some frequencies a discernable difference in signal strengths is shown between the connective tissue, the carcass surface and the hide tissue, in most cases this difference is quite small and in one case is inconsistent with other results in terms of the relationship between the 3 test site regions of hide, connective tissue and muscle tissue. It is therefore unlikely that there would be sufficient dissemination of the signals at a consistent level to be useful in discriminating the connective tissue from surrounding tissues with this sensing method.

Fluorescent Spectroscopy

A number of the samples taken during the experimental trial were sent to the Biophotonics and Laser Laboratory at the University of Queensland (UQ) in order that fluorescent spectroscopy could be carried out on the tissue. The samples were sent frozen to UQ where they were further prepared (sectioned into thin slices) to be suitable for the fluorescence work.

The results from the fluorescence work carried out as well as subsequent discussions with the researchers from UQ indicate that it may be possible to discriminate connective tissue from surrounding tissue types by means of the fluorescence method, based on the samples tested. In particular, there appears to be a wide enough spread of relative fluorescence intensities between three of the major tissue types (hide, connective tissue and muscle tissue) to allow effective discrimination.

It should be noted however, that there may be other tissue types that, for practical purposes, are more difficult or even impossible to discriminate using this method. For example, the elastic type tissue found in sample # 5:16 has a 'signature' close to that of connective tissue and may prove difficult to effectively discriminate from the connective tissue. Further research must be carried out to establish whether this method can effectively and consistently discriminate between all the tissue types that are likely to be encountered near the hide interface connective tissue.

Some issues will require further investigation to determine if this method can be practically applied. The operation of a sensor or probe using this method would require the exclusion of external light, at least locally where the sensor or probe is working and this may be quite difficult to achieve in a commercial abattoir processing environment. Another issue that needs to be addressed is the speed (sampling rate) at which the probe or sensor can operate. This speed needs to be sufficiently high to ensure that operation in real time is achievable.

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1. INTRODUCTION

The detection of the interface between the carcass surface and the hide at removal will provide a sensing technology from which an automatic system for hide removal could be controlled.

Further development of a separation method to follow the interface position could result in a carcass with superior surface appearance and reduce the incidence of cuts and nicks that downgrade the value of the hide and primal cuts.

The development of a sensing system to identify the carcass hide seam line may also provide an approach on how the break-down of primal cuts could occur. This could be the identification of the interface between similar tissues, for example the longitudinal seams between the silverside, topside and thick flank, or interfaces between carcass components with different characteristics such as fat, muscle, and bone.

Milestone 1 detailed the work carried out to determine the location and size of sample areas of a beef carcass for testing and further experimentation as well as determining the sensing methods that would be applied. Additionally, milestone one, determined the need to combine the reporting of work for milestones two and three into one final report to better reflect the actual progression of work on the project.

The work carried out in milestone two/three includes; carrying out an experimental trial on a beef carcass to determine the effectiveness of selected sensing methods; collection of samples from various areas of the carcass and; further testing and analysis of the samples and development of the results.

1.1 DEFINITION

A comprehensive study of the histology of bovine hide and subcutaneous tissue at various locations on the body is beyond the scope of this project; however it may be useful to look at a typical section of hide and underlying tissue to better understand the general arrangement. Figure 1 shows an enlarged sectional view of a representative hide/subcutaneous tissue sample. The interface connective tissue is clearly shown as a loose, fibrous area sandwiched between the reticular dermis of the hide and the underlying fat or muscle tissue. Depending on what area of the carcass the sample is taken from, the connective tissue may attach directly to other tissue types such as fat, hide or muscle. The thickness of the connective tissue also varies in different areas of the carcass. The connective tissue in this area is the interface requiring detection for this project.



Figure 1: View of hide and subcutaneous tissue.

2. EXPERIMENTAL TRIAL

An experimental trial was carried out on a 240kg Hereford Cross yearling steer. Two sensing methods were tested on the carcass during the trial and a third method was tested, at a later date, on samples taken from the carcass. Some of the samples were also sent for collagen concentration analysis to determine the collagen content of various tissue types.

The sensing methods investigated were;

- Low Voltage Radio Spectrum Transmissibility
- Thermal Imaging
- Fluorescent Spectroscopy

The trial was carried out on 06/12/2005 at the Food Science Australia Cannon Hill laboratory. The animal was stunned and bled and then positioned, inverted, in a bed dressing cradle for further processing.

Self-adhesive sample patches were positioned on the carcass at the sample site areas identified from milestone one. Figure 2 shows the carcass, inverted in the bed dressing cradle, with some of the sample patches in place.



Figure 2: Sample patches positioned on carcass

Some difficulty was experienced in getting the patches to stick effectively to the hide, particularly when it was being handled during cutting out of the patch area. Eventually it was decided to use some supa-glue between the patch and hide to achieve suitable adhesion. The sample areas were cut out to a suitable depth to ensure each sample contained the full depth of hide, connective tissue and some subcutaneous tissue of muscle or fat. After the sample areas were removed from the carcass they were then cut up into the smaller 4cm x 4cm squares and placed in resealable bags for freezer storage.

Figure 3 below shows one of the 4cm x 4cm individual samples with the hide peeled away from the subcutaneous muscle tissue and revealing the connective tissue still partly attached to hide and muscle.



Figure 3: Individual sample #7:5.

2.1 THERMAL IMAGING

A SAT model HY6700 thermal imaging (infrared) camera was used for the trial to record images during separation of the hide from the carcass. The camera is a high resolution type with a spectral range of 8 to 14 microns and spectral resolution of 1.3 milliradians (mrad)/pixel. This type of camera is sometimes used in the medical and veterinary fields to diagnose muscle, bone or circulation problems.



Figure 4: SAT HY6700 Thermal Camera

2.1.1 METHOD

Immediately after the animal was slaughtered and placed in the bed dressing cradle, the hide was opened and images were recorded with the thermal camera as the hide was removed and the hide/carcass interface area was exposed. Images were also recorded while using a chilled 40mm diameter round aluminium bar in contact with the outside (hair side) surface of the hide as a heat sink to draw heat from the hide.

2.1.2 RESULTS

Figure 5 shows a thermogram taken as the hide was removed from an area of the carcass near the brisket. The scale on the right side of the thermogram shows the set range of temperatures within the thermogram (27.95'C to 35.27'C) while the colour bargraph shows the change of colour corresponding to the temperature range. The highest temperatures of the carcass within the image area (white areas) can be seen around the separation line of carcass/hide and spread across some of the nearby skinned areas of the carcass. The dotted line in figure 5 shows the approximate location of the separation line. The thermogram is slightly blurred as it was taken at the limit of the cameras' focal range. This thermogram can be compared with the standard colour image in Figure 6, which was taken of the same area at approximately the same time from a slightly different viewpoint.

Figure 7 shows a thermogram of the same general view as Figure 5 after a 16 second interval (the view was panned slightly for this shot). The separation line of the hide is seen more clearly in this thermogram, possibly due to the time delay allowing a slight cooling on the exposed, skinned surfaces. Typically, the difference in temperatures between the warmer area of the separation line and the cooler surrounding tissue is in the order of 1 to 2 degrees Celsius.



Figure 5: Thermogram of hide being removed showing interface line



Figure 6: Standard image for comparison with figs. 3&5.



Figure 7: Thermogram after 16 second delay showing interface line.

In order to determine if the thermal appearance of the separation line could be further enhanced, a chilled aluminium bar (chilled to approximately -20'C) was placed in contact with the external surface of the hide, close to the area where the hide was being removed (separation line). It was hoped that the bar would act as a suitable heat sink to help further contrast the thermogram of the separation line. Figures 8 to 11 show a series of thermograms taken of the hide being removed with an average time interval of approximately 45 seconds between each image. The chilled bar is held in place against (behind) the hide in each of the thermograms and the heat sink effect of the bar can be seen as the removed hide rapidly decreases in temperature, showing areas of darker colour, during the series of thermograms. The bar appears black in the thermograms due to its low temperature. Figure 12 is a standard colour image included for comparison with figures 8 to 11.

The separation line is just visible in Figure 8 as a faint white line, however the line does not appear to be enhanced by the chilling effect on the hide from the thermal data as seen in Figures 9 to 11. Instead, the separation line is more difficult to see in those thermograms with the visible temperature contrast of the separation line dissipating into the surrounding tissue.



Figure 8: Thermogram with heat sink in place.



Figure 9: Hide being removed, some chilled hide now visible.



Figure 10: Additional hide removed, larger chilled area of hide visible.



Figure 11: Further chilled hide removed, but no enhancement of separation line.



Figure 12: Standard image showing general position of chilled bar.

2.1.3 CONCLUSION

From the results of thermal imaging carried out during the experimental trial it is clear that, at least in some circumstances, a visible line can be seen in thermograms at the point where the hide separates from the carcass (separation line). It is not clear why this line is visible or whether the connective tissue itself has some unique properties that might cause this effect. Neither is it possible to arrive at a conclusion based upon the limited data within this study. However, there are a number of possible reasons/mechanisms that are worth considering. It is also conceivable that more than one of the possible explanations that are listed below may be in operation at the same time.

Reference should also be made to Section 3 - Additional Thermal Images.

- The hide, being a good thermal insulator, will have an insulating effect on the underlying carcass tissue and as the hide is removed, a certain amount of surface cooling (possibly enhanced by evaporative cooling due to moisture on the surface) occurs quite rapidly on the skinned surface of the carcass and the inside (skinned) surface of the hide. Therefore, the hotter area viewed at the separation line may simply be a view of the edge of a slightly warmer area, thermally protected by the attached hide, where the surface cooling as described above has not yet occurred.
- 2. The connective tissue may have some unique thermally conductive/reflective properties (unlikely, as the already severed tissue on the carcass should also be displaying higher temperatures)
- 3. During the trial, it was noted that applying more pulling force to the hide appeared to increase the appearance (intensity) of the separation line so that it became more visible. It is possible that due to the porous nature of the connective tissue, the stretching that occurs in the tissue when the hide is pulled away from the carcass might allow an increased area of heat (core heat) to become thermally 'visible', whereas it would normally be obscured by the relaxed hide and connective tissue
- 4. Similar to above, when the hide is pulled, a certain amount of energy is transferred through the connective tissue resulting in a small increase in heat (through internal friction) as the tissue is stretched.
- 5. Similar to 3 and 4 above, when the hide is pulled, some of the connective tissue as well as small blood capillaries are pulled out of underlying fat or muscle tissue mass which has a higher core temperature, thus briefly exposing the hotter tissue. When enough force is applied or if a knife cut is made, the tissue is severed and some retracts back into the fat or muscle mass.

Chilling the outside of the hide in order to achieve a greater temperature differential between the hide and the underlying carcass tissue and thus improved thermal contrast of the separation line does not appear effective.

2.2 LOW VOLTAGE RADIO SPECTRUM TRANSMISSIBILITY

An experiment was set up to determine whether it is possible to accurately discriminate between different tissue types based on the interpretation of a radio signal transmitted through the different tissues. For example, if the connective tissue at the hide interface was shown to be a better transmission medium than the surrounding tissue, then it may be possible to discriminate the two.

In simple terms, this means injecting a small electrical signal into the body of the animal and then measuring the strength of that signal at different points on the body (hide, connective tissue, muscle tissue). The greater the difference in signal strengths at those points, the better the chance of discriminating between the tissues.

A radio frequency generator was connected to two probes (comb shaped) and a series of relatively low amplitude sine waves at different frequencies were injected into the body of the carcass.

The received signal was detected on a modified knife touching the surface of the carcass, with the signal return path going through a multi-pronged probe inserted into the bulk of the body not far from the knife. A Rohde & Schwarz FSH3 spectrum analyser was used to determine the strength of the returned signal.

Figure 13 shows the arrangement of the signal injection and spectrum analyser probes during the trial.

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Figure 13: General view of signal injection and spectrum analyser probes during trial.

2.2.1 METHOD

The probe for the spectrum analyser was manufactured from the metal blade of a 150mm boning knife. The blade was covered with an insulating material so that only about 1.5mm of the sharpened edge of the blade was exposed along its length. Using this type of design, it was possible to selectively probe the thin connective tissue layer between the hide and subcutaneous tissue. The amplitude of the injected signal was fixed at 900 millivolts (AC) peak to peak. In order to determine an optimal transmission frequency, six frequencies were selected. They were;

- 200 kHz
- 600 kHz
- 2 MHz
- 6 MHz
- 21 MHz
- 75 MHz

Electrical signals were injected into the carcass and the blade type probe was brought into contact with the carcass to measure the relative signal strength at that particular point of the carcass. In order to determine if the connective tissue at the hide/carcass interface could be discriminated, the probe was placed in three different areas for each of the frequencies selected. The probe was first touched to the skinned carcass surface, then to the connective tissue at the hide/carcass interface and finally onto the inside of the skinned hide surface. At each point the signal strength as measured by the spectrum analyser was recorded. All measurements were taken from the same general area of the carcass. Any significant difference between the signal strengths as measured at the three different locations (hide, interface, and carcass) would indicate a potential means of detecting the interface.

2.2.2 RESULTS

This section shows the resultant plots for each of the frequency ranges investigated. The vertical axis of the plot indicates the signal strength while the horizontal axis shows the frequency. At each selected frequency the signal is measured at three different points on the carcass – the inside surface of the skinned hide, the connective tissue at the interface and the skinned surface of the carcass.

Figures 14, 15 and 16 shown below are spectrum analyser plots of the frequency response at 200 kHz. Figure 14 shows the plot taken from the measurement on the inside surface of the hide, displaying signal strength of -84dBV. Figure 15 shows the plot from the connective tissue at the hide/carcass interface with signal strength of -80dBV. Figure 16 shows the plot from the skinned surface of the carcass with signal strength of -84dBV.



Figure 14: 200 kHz plot from inside hide.



Figure 15: 200 kHz plot from connective tissue.



Figure 16: 200 kHz plot from carcass surface.

The above plots at the 200 kHz frequency show that there is a small, but measurable, difference between the connective tissue and the other surfaces, however, the signal strengths recorded from the hide and the carcass (Figs. 14 & 16) are so similar that it would be very difficult to distinguish between them.

Figures 17, 18 and 19 show the spectrum analyser plots at 600 kHz. Figure 17 is the plot from inside the hide showing signal strength of 87dBV. Figure 18 is the plot from the connective tissue with signal strength of 83dBV. Figure19 is the plot from the carcass surface showing signal strength of 84dBV.



Figure 17: 600 kHz plot from inside hide.



Figure 18: 600 kHz plot from connective tissue.



Figure 19: 600 kHz plot from carcass surface.

The 600 kHz set of plots (above) again show a small measurable difference between peak signal strengths however the values from the carcass surface and the connective tissue are very close and again, would cause difficulties with discrimination. It should also be noted that background noise at this frequency potentially infringes on the target signal. Figures 20, 21 and 22 show the spectrum analyser plots at 2 MHz. Figure 20 is the plot from inside the hide with signal strength of 80dBV. Figure 21 is the plot of the connective tissue with signal strength of 79dBV. Figure 22 is the plot from carcass surface showing signal strength of 83dBV.



Figure 20: 2 MHz plot from inside hide.



Figure 21: 2 MHz plot of connective tissue.



Figure 22: 2 MHz plot from carcass surface.

The 2 MHz set of plots (above) again indicate only small differences in signal strengths, particularly between the inside of the hide and the connective tissue interface.

Figures 23, 24 and 25 show the spectrum analyser plots at 6 MHz. Figure 23 is the plot from inside the hide with signal strength of 80dBV. Figure 24 is the plot of the connective tissue with signal strength of 79dBV. Figure 25 is the plot from carcass surface showing signal strength of 84dBV.



Figure 23: 6 MHz plot from inside hide.



Figure 24: 6 MHz plot of connective tissue.



Figure 25: 6 MHz plot from carcass surface.

The 6 MHz plots (above) are very similar to the previous set of plots showing very minimal difference in signal strength between inside of hide and the connective tissue.

Figures 26, 27 and 28 show the spectrum analyser plots at 21MHz. Figure 26 is the plot from inside the hide showing signal strength of 88dBV. Figure 27 is the plot from the connective tissue with signal strength of 93dBV. Figure28 is the plot from the carcass surface showing signal strength of 77dBV.



Figure 26: 21 MHz plot from inside hide.



Figure 27: 21 MHz plot from connective tissue.



Figure 28: 21 MHz plot from carcass surface.

The 21 MHz plots appear to show some relative difference in signal strength, particularly between the carcass surface and the connective tissue. This set of plots however, seem inconsistent with all other plot sets in regard to the relatively low signal strength of the connective tissue compared to the other two test sites and it is possible that there may have been poor contact between the probe and the connective tissue in this case.

Figures 29, 30 and 31 show the spectrum analyser plots at 75MHz. Figure 29 is the plot from inside the hide showing signal strength of 97dBV. Figure 30 is the plot from the connective tissue with signal strength of 94dBV. Figure31 is the plot from the carcass surface showing signal strength of 93dBV.



Figure 29: 75 MHz plot from inside hide.



Figure 30: 75 MHz plot of connective tissue.



Figure 31: 75 MHz plot from carcass surface.

The 75 MHz set of plots show that there is very little difference in signal strength between the connective tissue and the carcass surface. At this frequency there is also a considerable problem with background noise infringing on the target signal.

2.2.3 CONCLUSION

The results of the low voltage radio spectrum transmissibility tests indicate that at some frequencies a discernable difference in signal strengths is shown between the connective tissue, the carcass surface, and the hide tissue. In most cases however, this difference is quite small and in one case (21 MHz) is inconsistent with other results in terms of the relationship between the 3 test site regions.

Frequency	Test site peak signal strength (dBV)			
	Hide	Connective Tissue	Carcass Surface	
200 KHz	-84	-80	-84	
600 KHz	-87	-83	-84	
2 MHz	-80	-79	-83	
6 MHz	-80	-79	-84	
21 MHz	-88	-93	-77	
75 MHz	-97	-94	-93	

The table shown below summarises the results of the frequency ranges from each of the three test site regions on the carcass.

Figure 32: Table of results from spectrum analyser plots.

The transmitted signal is, in itself, reasonably strong and could be extracted to determine if a sensor is in contact with the carcass or not, that is, the carcass in its entirety can be regarded as a moderately good transmission medium at frequencies below about 30 MHz. For frequencies above this point, filtering and tuning becomes more important when determining a transmitted signal.

It may be concluded therefore, that on the basis of these tests, there would probably not be sufficient dissemination of the signals at a consistent level to be useful in discriminating the connective tissue from surrounding tissues.

2.3 FLUORESCENT SPECTROSCOPY

Steady State Fluorescence Spectroscopy is a technique that is widely used in analytical sciences. It involves exciting a sample with light of a certain frequency. If the energy of a photon of this light corresponds to the energy gap between a ground and excited state of an electron associated with a molecule in the sample it will be absorbed. Before the electron decays back to its ground state by emitting a photon it loses energy via vibration and rotational modes. This is why the emitted photon is generally of a lower energy than the absorbed photon. This emitted photon is what is classed as the fluorescence.

A photoluminescence (PL) scan involves exciting the sample with a single wavelength of light and detecting the fluorescence at a range of different wavelengths. On the other hand a photoluminescence excitation (PLE) scan involves exciting the sample at a range of different wavelengths and detecting at a single wavelength. If you have a number of either of these scans you can compile a matrix plot. This is a 3-D plot that shows the intensity of fluorescence as a function of excitation and emission wavelength.

A number of the samples taken during the experimental trial were sent to the Biophotonics and Laser Laboratory at the University of Queensland (UQ) in order that fluorescent spectroscopy could be carried out on the tissue. The samples were sent frozen to UQ where they were further prepared (sectioned into thin slices) to be suitable for the fluorescence work.

The samples that were scanned were sample numbers; 3:10, 4:17, 5:16, 6:17, 7:21. As well as these five samples, a further sample of isolated (predominately) connective tissue taken from the forequarter region of the carcass during the experimental trial was scanned. For reference to the exact position of the samples on the carcass, refer to figures 33 & 34.



Figure 33: Position of sample areas on carcass.

1:1	1:2	1:3	1:4	1:5	1:6
1:7	1:8	1:9	1:10	1:11	1:12
1:13	1:14	1:15	1:16	1:17	1:18
	/			/	
1:19	1:20	1:21	1:22	1:23	1:24

Figure 34: Layout of individual samples on sample patch.

Each sample, as sent, generally consisted of a sandwiched arrangement of three components: the hide, connective tissue and subcutaneous tissue. The three components of the samples were then separated and the photoluminescence (PL) spectra of each component was recorded. The PL spectra of the separate isolated connective tissue was also recorded.

Figures 35, 36 and 37 shown below are matrix scans of, respectively, dermal tissue (hide), connective tissue and muscle tissue. The three scans indicate the differences between the three tissue types in terms of excitation and emission wavelengths (measured in nanometres) and the intensity of fluorescence (measured in million counts per second). The peaks shown in the scans represent the spectral position of certain fluorophores, mainly those associated with collagen, elastin and amino acids. Therefore, the spectral position and intensity of the peaks for each of the sample components (hide, connective tissue and muscle tissue) tend to identify that component, much as a signature.



Figure 35: Matrix scan of hide tissue.

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Figure 36: Matrix scan of connective tissue.



Figure 37: Matrix scan of muscle tissue.

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Another subcutaneous tissue type that was scanned came from sample #5:16. In the report in the Appendix, it is noted that this tissue is located below the connective tissue layer and is somewhat elastic in nature. This tissue is actually part of a broad fibrous sheet called the *Tunica Flava Abdominis.* This elastic tissue is similar to ligament type tissue and assists the muscles in supporting the weight of the viscera. The matrix scan for this tissue type is shown in figure 38.



Figure 38: Matrix scan of elastic tissue from sample #5:16.

2.3.1 CONCLUSION

The results from the fluorescence work carried out as well as subsequent discussions with researchers from UQ indicate that it may be possible to discriminate connective tissue from surrounding tissue types by means of the fluorescence method, based on the samples tested. In particular, there appears to be a wide enough spread of relative fluorescence intensities between three of the major tissue types (hide, connective tissue and muscle tissue) to allow effective discrimination.

It should be noted however, that there may be other tissue types that, for practical purposes, are more difficult or even impossible to discriminate using this method. For example, the elastic type tissue found in sample # 5:16 has a 'signature' close to that of connective tissue and may prove difficult to effectively discriminate from the connective tissue (Refer also to Section 2.4 Collagen Concentration). Further research must be carried out to establish whether this

method can effectively and consistently discriminate between all the tissue types that are likely to be encountered near the hide interface connective tissue.

Some issues will require further investigation to determine if this method can be practically applied. The operation of a sensor or probe using this method would require the exclusion of external light, at least locally where the sensor or probe is working and this may be quite difficult to achieve in a commercial abattoir processing environment. Another issue that needs to be addressed is the speed (sampling rate) at which the probe or sensor can operate. This speed needs to be sufficiently high to ensure that operation in real time is achievable.

2.4 COLLAGEN CONCENTRATION

The most abundant protein in animals is collagen. The predominant types of collagen found in the dermal, connective and muscle tissue are types I and III collagen. While determining the possible sensing methods to apply to the carcass during the experimental trial as well as to the samples collected during the trial, it was thought to be useful to obtain some information about the collagen content of the tissue types encountered at and near the hide interface, particularly since collagen is a major component in these tissues and is known to have particular properties such as fluorophores for example, that cause it to fluoresce under the right conditions.

A collagen concentration analysis was carried out on samples from two of the sample sites from the experimental trial as well as the separate sample of isolated connective tissue taken from the forequarter area of the carcass. The samples were prepared by carefully isolating the dermal tissue (hide) from the connective tissue. The hide from each sample was then processed using standard laboratory practice to determine total collagen content. The connective tissue was isolated from the subcutaneous tissue and tested along with the separate connective tissue sample. The table below shows the results of the collagen content analysis.

	Sample	% collagen (wet wt)	% collagen (dry wt)
2.6	Connective tissue	10.24	-
2.6	Hide	13.80	37.92
5.10 elastic	Connective tissue layer covering	20.92	-
5.10 Abdor	Elastic tissue (<i>Tunica Flava</i> ninis)	9.78	26.87
5.10	Connective tissue closest to hide	12.36	-
5.10	Hide	11.90	32.68
Should sample	der connective tissue (separate e)	7.79	-

Figure 39: Table of results for collagen content.

Included in the table are the results for the elastic type tissue (*Tunica Flava Abdominis*). This tissue was also isolated from surrounding tissues for testing. From the collagen concentration analysis, the percentage of collagen shown is the total of all types of collagen present however, due to the tissue types sampled, would predominantly be collagen types I and III, except for the elastic tissue, which would predominantly be type II.

Figures for the dry weight percentage of connective tissue were not obtainable due to the very small amounts of tissue available from individual samples. Also, for this test it was decided not to try and process underlying fat/muscle tissues due to the problems of fats interfering with the preparation/analysis process.

Figure 40 below shows sample #5:10 separated into its' four major components prior to testing. Clockwise from the top left of the image, the components are; 1) Connective tissue covering elastic tissue. 2) Connective tissue closest to hide. 3) Dermal tissue (hide). 4) Elastic Tissue. Note that the elastic tissue shown in this image is the same type tissue (*Tunica Flava Abdominis*) that was tested in the fluorescence trial. It should also be noted that this tissue showed a relatively high collagen content, similar to levels found in the connective tissue. This information supports the need for further investigation into tissue characterisation and differentiation, particularly if fluorescence spectroscopy is to be developed for interface detection.



Figure 40: The four components of sample #5:10.

3. ADDITIONAL THERMAL IMAGES

During the course of the project, an opportunity arose to carry out some further thermal imaging within a commercial abattoir environment. On 01/02/2006, thermal images were taken of the hide pulling operation at a commercial abattoir. This plant uses an industry standard downward hide pulling machine with 2 operators utilised to attach chains to the hide and assist separation with air knives as the hide is being removed. This minimises any plucking of fat or muscle and achieves a better finish on the carcass. Thermographic data was captured by 2 means during the hide pull procedure. Still images were taken at short intervals and simultaneous video footage was recorded live from the thermal camera.

The images shown below (Figures 41 to 46) are a sequence of thermograms taken during the hide pull operation. These thermograms, particularly figures 42 to 46, clearly show the separation line of the hide as a consistently higher temperature zone during the hide removal process.

The first thermogram in the sequence (Figure 41) shows the carcass a few seconds before the hide pull begins with the separation line barely visible. The maximum temperature at the interface in this image is 34.15'C. The next five images (Figs. 42 to 46) follow the separation line of the hide as it is being removed from the carcass. In these thermograms the maximum temperature at the interface is considerably higher at between 34.55'C to 38.37'C maximum temperatures.



Figure 41: Thermogram before hide pull starts.



Figure 42: Thermogram as hide pull is started. Note higher temperature at interface. Elapsed time (from Fig. 41) 6 seconds.



Figure 43: Thermogram as hide pull progresses. Total elapsed time 9 seconds.



Figure 44: Thermogram as hide pull progresses. Total elapsed time 12 seconds.

Interface 40.95°C - 40 - 38 - 36 - 34 - 32 - 30 - 28 - 27.87°C | 7.4

Figure 45: Thermogram as hide pull progresses. Total elapsed time 14 seconds.



Figure 46: Thermogram as hide pull progresses. Total elapsed time 20 seconds.

The thermograms above were also compared to the live thermal video footage taken at the same time and it was found that the higher temperature visible at the separation line of the hide was related to the action of the hide separation. That is, the higher temperatures are associated with (and likely caused by) the dislocation of the hide from the carcass. The video footage clearly shows that there is an increase in temperature at the interface *during* the hide pull and that this additional heat rapidly dissipates immediately after the dislocation of the hide has occurred, giving the impression of a moving 'heat line' when viewed with a thermal camera.

The actual amount of temperature rise at the interface during the hide pull appears to be variable and has not been fully quantified in this study. However, from the information gathered at the time, the temperature rise would seem to be in the order of several degrees.

At this stage, it is not known for certain what causes the increased heat at the interface during hide pull, however, as also mentioned in Section 2.1.3, one of the more likely causes is heat generated through internal friction in the stretched

and breaking connective tissues. The heat generated in this manner is most likely linked to "hysteresivity" which is a term used to describe the mechanical coupling between energy dissipative forces and tissue elastic properties. In recent times, considerable research has been carried out in areas such as lung tissue elasticity where frictional stress (hysteresivity or equivalently the structural damping coefficient - Fredberg JJ, Stamenovic D., 1989) has been quantified almost invariably as between 0.1 to 0.2 of the elastic stress therefore, from the peak elastic strain energy that is stored during cyclic deformation, 10 to 20% of the elastic energy is lost as friction and expressed as heat. There is evidence that this fixed relationship applies to some other tissue and cell types also.

When hysteresivity is considered, it is not unreasonable to assume that some, if not all of the additional heat evident at the interface area during hide pull is a result of frictional stresses. However, assuming this is true, it is still unclear whether all tissue types near the interface area would behave in the same manner. Further research is required, for example, to investigate whether a hide pull that encountered problems and started tearing through muscle tissue would display the same level of increased heat at the interface where the muscle was being torn.

4. CONCLUSION

Three potential sensing methods have been applied to determine the suitability of the different characteristics of the hide/carcass interface tissue for detection. Those methods were:

- Thermal Imaging
- Low Voltage Radio Spectrum Transmissibility
- Fluorescent Spectroscopy

Thermal Imaging

From the results of thermal imaging carried out during the experimental trial it is clear that, at least in some circumstances, a visible line can be seen in thermograms at the point where the hide separates from the carcass (separation line). It is not clear why this line is visible or whether the connective tissue itself has some unique properties that might cause this effect. Neither is it possible to arrive at a conclusion based upon the limited data within this study. However, there are a number of possible reasons/mechanisms that are worth considering. It is also conceivable that more than one of the possible explanations that are listed below may be in operation at the same time.

- The hide, being a good thermal insulator, will have an insulating effect on the underlying carcass tissue and as the hide is removed, a certain amount of surface cooling (possibly enhanced by evaporative cooling due to moisture on the surface) occurs quite rapidly on the skinned surface of the carcass and the inside (skinned) surface of the hide. Therefore, the hotter area viewed at the separation line may simply be a view of the edge of a slightly warmer area, thermally protected by the attached hide, where the surface cooling as described above has not yet occurred.
- 2. The connective tissue may have some unique thermally conductive/reflective properties (unlikely, as the already severed tissue on the carcass should also be displaying higher temperatures)
- 3. During the trial, it was noted that applying more pulling force to the hide appeared to increase the appearance (intensity) of the separation line so that it became more visible. It is possible that due to the porous nature of the connective tissue, the stretching that occurs in the tissue when the hide is pulled away from the carcass might allow an increased area of heat (core heat) to become thermally 'visible', whereas it would normally be obscured by the relaxed hide and connective tissue
- 4. Similar to above, when the hide is pulled, a certain amount of energy is transferred through the connective tissue resulting in a small increase in heat (through internal friction) as the tissue is stretched.
- 5. Similar to 3 and 4 above, when the hide is pulled, some of the connective tissue as well as small blood capillaries are pulled out of underlying fat or muscle tissue mass which has a higher core temperature, thus briefly exposing the hotter tissue. When enough force is applied or if a knife cut is made, the tissue is severed and some retracts back into the fat or muscle mass.

Chilling the outside of the hide in order to achieve a greater temperature differential between the hide and the underlying carcass tissue and thus improved thermal contrast of the separation line does not appear effective.

Additional thermal images taken of the hide pull procedure at the abattoir, under very different environmental conditions (lighting, temperature, carcass orientation, etc.), confirmed the presence of the thermally visible line at the hide interface. Analysis of the thermograms and the thermal video footage taken at the same time, also show the presence of an additional heat zone at the hide interface during the hide pull. This heat zone may be several degrees above the expected temperature at the separation line of the hide and is short lived (from observation, less than 1 second) dissipating rapidly away from the separation line during hide pull or as soon as the separation process is halted. The cause or causes of this additional heat is not known for certain at this stage, although it is likely to be linked to hysteresivity where some of the elastic energy present in the interface material is expressed as heat, through frictional stress.

When hysteresivity is considered, it is not unreasonable to assume that some, if not all of the additional heat evident at the interface area during hide pull is a result of frictional stresses. However, assuming this is true, it is still unclear whether all tissue types near the interface area would behave in the same manner. Further research is required, for example, to investigate whether a hide pull that encountered problems and started tearing through muscle tissue would display the same level of increased heat at the interface where the muscle was being torn.

Low Voltage Radio Spectrum Transmissibility

The results of the low voltage radio spectrum transmissibility tests indicate that at some frequencies a discernable difference in signal strengths is shown between the connective tissue, the carcass surface, and the hide tissue. In most cases however, this difference is quite small and in one case (21 MHz) is inconsistent with other results in terms of the relationship between the 3 test site regions.

The transmitted signal is, in itself, reasonably strong and could be extracted to determine if a sensor is in contact with the carcass or not, that is, the carcass in its entirety can be regarded as a moderately good transmission medium at frequencies below about 30 MHz. For frequencies above this point, filtering and tuning becomes more important when determining a transmitted signal.

It may be concluded therefore, that on the basis of these tests, there would probably not be sufficient dissemination of the signals at a consistent level to be useful in discriminating the connective tissue from surrounding tissues.

Fluorescent Spectroscopy

The results from the fluorescence work carried out as well as subsequent discussions with the researchers from UQ indicate that it may be possible to discriminate connective tissue from surrounding tissue types by means of the fluorescence method, based on the samples tested. In particular, there appears to be a wide enough spread of relative fluorescence intensities between three of the major tissue types (hide, connective tissue and muscle tissue) to allow effective discrimination.

It should be noted however, that there may be other tissue types that, for practical purposes, are more difficult or even impossible to discriminate using this method. For example, the elastic type tissue found in sample # 5:16 has a 'signature' close to that of connective tissue and may prove difficult to effectively discriminate from the connective tissue. Further research must be carried out to establish whether this method can effectively and consistently discriminate between all the tissue types that are likely to be encountered near the hide interface connective tissue.

Some issues will require further investigation to determine if this method can be practically applied. The operation of a sensor or probe using this method would require the exclusion of external light, at least locally where the sensor or probe is working and this may be quite difficult to achieve in a commercial abattoir processing environment. Another issue that needs to be addressed is the speed (sampling rate) at which the probe or sensor can operate. This speed needs to be sufficiently high to ensure that operation in real time is achievable.

5. REFERENCES

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