APPLICATION OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY TO ESTIMATE POST MORTEM INTERVAL

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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Estimating the post mortem interval of human remains is extremely important in death scene investigations. Currently, the rate of decomposition is gaged by a number of techniques, such as insect development, a function in part of environmental and weather conclusions. However, there are limitations with the process that prevent more accurate assessments. Near-infrared reflectance spectroscopy may provide complimentary and supportive estimates of the post mortem interval. The goal of this research was to determine if near-infrared reflectance spectroscopy (NIRS) could detect changes in decomposing skin of feral swine as a model system. NIRS spectra were obtained from swine skin samples exposed to natural elements (daily temperature, precipitation, humidity and solar radiation) on the campus of Texas A&M University during warm (July) and cool (February) seasons. Linear regression analysis of spectral data by sample age and state of decomposition were found to be highly correlated (R^2=0.8749) during the warm period exposure. The same regression analysis of spectral data taken during the cool exposure months produced an R^2 of 0.812.

NOMENCLATURE

PMI	Post Mortem Interval
NIRS	Near-Infrared Reflectance Spectroscopy

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CHAPTER I

INTRODUCTION

Improving upon the current methods for estimating post mortem interval

The need for a more accurate, and statistically proven way to estimated human PMI is becoming more of a necessity by the day. The idea of determining a PMI of human remains is not new to the field of forensic sciences (Di Miao and Di Miao 1993). Estimates of PMI rule out or narrow down the field of possible suspects in an investigation and are crucial in any criminal case. Algor mortis, rigor mortis, and livor mortis are examples of physiological changes that occur to a human body post mortem. These post mortem stages usually provide the basis from which PMI estimates can be made (Kaliszan et al. 2009). A majority of the current techniques employ the analysis of analytes that can contribute information up to a five-day period of time, after which, it can no longer be used. Beyond five days, the remaining resources for PMI estimation become reduced as well as lack the desired precision.

Problems with current techniques

Oftentimes, it falls on the shoulders of an entomologist to observe and collect the insect evidence and compare it to weather data that was taken in close proximity to where the body was found. Then by comparing calculated insect developmental time to the evidence timeline, a formal estimate can be made as to when the body was most likely colonized by a specific insect (Tomberlin et al. 2011). Although this way of analyzing, and developing a time scale of when the body was actually colonized is currently very useful, there are flaws in the process and fundamentals that prevent a more accurate assessment of when an individual actually died. The

major issue has to do with the gap between death and insect colonization on a corpse. With entomology, it is impossible to say how long this time gap actually was, and thus, it is impossible to definitively say when a person died; but rather make an educated estimate on when the body was colonized by insects (Villet et al. 2011**).** This problem will hopefully be solved if it can be established that post-mortem changes in decomposing tissues are detectable by near-infrared spectroscopy.

Advantages of near-infrared spectroscopy

Near-infrared spectroscopy is a non-destructive and non-invasive technique to estimate human PMI and would allow for numerical and statistical values to be associated with the science behind it. This would permit the technique to be accepted in a legal setting (Daubert Standards), as well as help to further narrow down the broad set of other unknowns that are inevitably present in any crime scene investigation. NIRS works by utilizing molecular overtones and combination vibrations that circumvent the selection rules of quantum mechanics. This trait ultimately means that molecules cannot absorb as much energy in the near-infrared spectrum as they would be able to with mid-infrared radiation. This quality comes with multiple positive attributes. Samples require little to no preparation and the actual reading takes minimal time with no degradation of the sample. NIRS is also inexpensive, fast and reliable. Also, due to near-infrared's absorptive qualities, the readings produce bands that are exceptionally broad and require exceedingly accurate calibration samples to precisely analyze a specific sample. This quality is comparable to other types of spectroscopy, such a UV-visible and mid-IR. NIRS is also an up and coming technique that is being used in the medical field, including measurement of hemoglobin. I propose to eventually evaluate NIRS detectable changes over time in human hair

and swine hoof and skin samples to determine rates of change that could have forensic application in PMI estimation. For the purposes of this research, we decided to concentrate our efforts on the analysis of swine dermal tissue, to hopefully validate and prove the concept behind using NIRS for this specific application.

CHAPTER II

METHODS

Sample origin: Human hair samples that have never been dyed nor cosmetically treated in anyway other than normal shampoo and rinse/conditioner will be collected by a single barber upon consent of each hair donor (barber's clients) (IRB2014-0570). Samples will be collected during one business day and shipped overnight to the department of entomology, Texas A&M University to minimize the time from collection to testing. The samples will be identified only by number and thus be blind as to any identification of the donor. The legs of hunter-killed feral swine will provide samples of vertebrate skin and hoof samples for the study. Samples will be placed on ice and transported to the project site on the same day to minimize the time from death to experimental measurements. Samples will be placed in outdoor animal exclusion cages for observation of degradation and measurement.

Experiment Duration: The experiment will measure changes in sample exposure and degradation across a six-month timespan. Degradation will be photographed and optical measurements made daily for one week, then followed by seven more readings that are three days apart, and then with readings taken weekly throughout the remainder of the period. I will analyze and compile the results to determine how well correlated the NIRS instrument can produce readings that are comparable to specific ages of the samples.

Optical Measurements: Optical measurements will be made using an Ocean Optics model NIRQuest512 portable NIR spectrometer with optical scanning from 900-1700 nm using a 512-

element InGaAs array with NIR3 grating and SLIT-25. The instrument uses a tungsten halogen light source optimized for the VIS-NIR from 360-2400 nm. Sample readings will be made using the premium 400 um fiber optic reflection probe (VIS/NIR, 2m) equipped with WS-1 diffuse reflectance standard and with positioning optimized with use of a reflection probe holder (RPH-1) for 6.35 mm diameter probes. The probe consists of 6 illumination fibers in a tight bundle positioned around a central "read" fiber and is designed for specular or diffuse reflectance applications from surfaces, solutions, or powders. Unit operation is assisted with cross-platform spectroscopy operating software, and calibration is facilitated with nominal calibration files for WS-1 and STAN-SSH standards. Spectral data are recorded and stored in MS Excel file format, which allows for ease of data transfer and application to analytical tools. Multiple readings will be made daily for one week, then at 3-day intervals, and then weekly of leg tissue, hoof and human hair.

Sample spectra will be grouped by date and sample type and analyzed using Grahams and Unscrambler multivariate analysis software systems. Spectral data will be organized into a gradient of "age" periods from earliest days to longest monthly exposure to test for spectral differences by time. Difference spectra and principal component score plots will be used to visually illustrate differences in NIR spectra, and covariate analyses will be conducted using the Statistical Analysis System (SAS, Cary, NC) to define the best fitting models of change over time. In order to get the overall perspective of the data's change over time, it will be imperative to group a variety of sample readings together. This variety in sample groupings will help to distinguish the variables in our research, and help illustrate the effects of sample differences.

CHAPTER III

RESULTS

Our observations and analysis will focus on seasonal comparison of replicated skin readings taken by sample date from site A (leg pastern or proximal interphalangeal joint), on pig leg one in the warm versus cool season exposures. The results were astounding as it showed, with very high percentage of certainty, that NIRS is detecting changes in tissue decomposition over time. This grouping of spectra is only one of thousands that is necessary and possible for proving that NIRS can be used in such an application.

Each time readings were taken, a table was brought out to the cage, along with the laptop, NIRS equipment, and a camera was set on a tripod to properly document the progress of the experiment. Once initial pictures were taken of the samples, readings could then be taken. The process would begin at pig sample one, scan the hoof three times, then progress to scanning each of the shaved sample areas (A, B, and C) three times. This process was repeated until all twelve legs were read. At this point the outside hair board could be scanned by reading from sample one to sample twelve a total of three separate times. This allows for an average to be taken from the readings from each day, which improves accuracy and helps account of the minimal differences in probe placement and sample imperfections. All equipment then was shut down and put away in order to move inside. Once inside the control hair board could be obtained, which was kept high out of the way from being disturbed and in a room where the light and temperature is held constant, and begin setting up the NIRS equipment to take readings. The inside board was then read the same way as the outside board.

Readings of the dermal samples were taken every morning, usually early to mid-afternoon, for twenty-one days straight. This daily pattern started on February 7th, and is ongoing. On a date that has yet to be determined, it will become necessary to cut the portion of the pig legs off which contained reading sites B and C. This will need to happen due to the excessive decomposition of the more distal sites. It will become impossible to obtain accurate readings because of the compromised skin in these areas. Reading sites B and C are on sections of leg that fall on more meat and muscle that site A. Reading site A falls on top of mainly bone and some ligaments. For this reason, keeping reading site A will be more practical due to consistent trends of this specific area being less decomposed, and readable for extended periods of time. For these reasons, we suggest scanning the anatomical equivalent of this area, the ankles or wrists, when this technique is eventually applied to humans.

Linear regression analysis of spectral data by sample age and state of decomposition using Grahams software were found to be highly correlated (R^2=0.8749) during the warm season exposure period exposure (Figure 1). The same regression analysis of spectral data taken during the cool season exposure months produced an R^2 of 0.812 (Figure 2). Visual inspection of regression lines for each seasonal exposure (Figure 1 and 2) suggests that an even better fit may be obtained with non-linear regression to possibly better conform to apparent bends in the response curve.

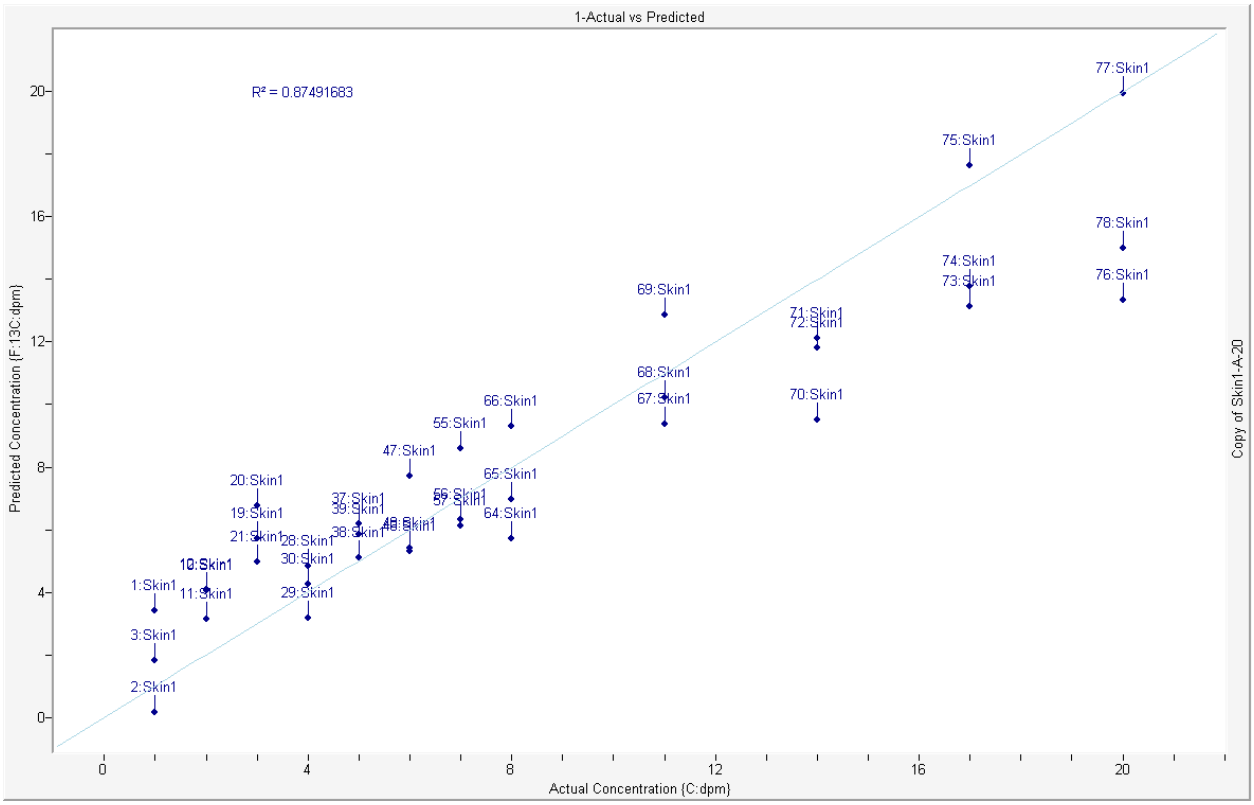

Figure 1. Linear regression analysis testing the association of NIRS spectra and sample age with readings taken from reading site A on the first leg sample only for the warm season exposure. Spectra included in this analysis were taken across a nineteen-day period, as the first seven readings were daily, followed by four more readings taken at 3-day intervals. Reading began on July 10th, which is represented by the first set of entries on the x-axis, and continued past the displayed set of analyzed data shown above.

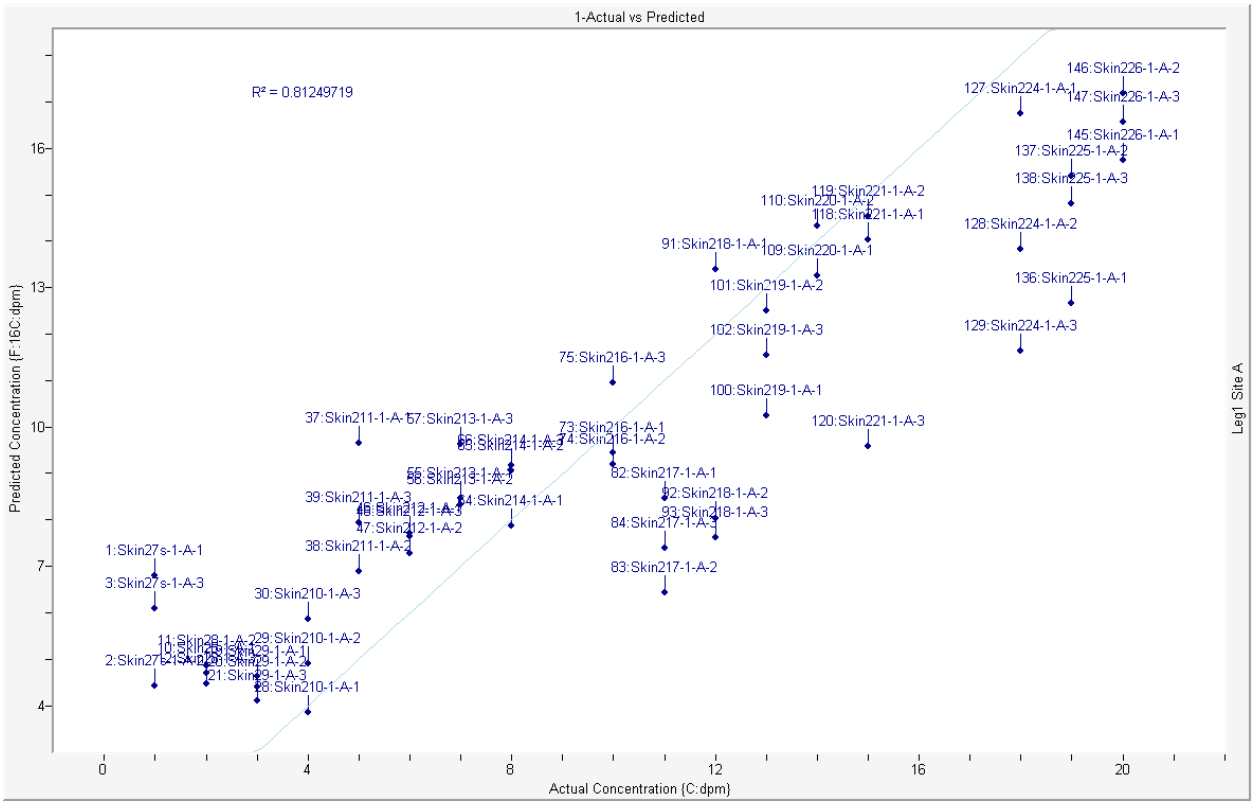

Figure 2. Linear regression analysis testing the association of NIRS spectra and sample age with readings taken from reading site A on the first leg sample only for the cool season exposure. Spectra included in this analysis were taken across a nineteen-day period with gaps in the daily trend, which can be attributed to the weather. Reading began on February 7th, which is represented by the first set of entries on the x-axis, and continued past the displayed set of analyzed data shown above.

An examination of spectra from 950-1700 nm layered by age of sample read (Figures 3 and 4) provides a basis for interpreting how the spectra were changing as time progresses. It is important to know, with as much precision as possible, what is changing and why it might be altered from the sample's original state. What increasingly became obvious was how the spectra tended to decrease in absorbance across the entire spectral range, and how this decline seemed to

be happening in increments that were related to sample age. This lends itself to the thought that the samples are not able to absorb as much light as time progresses; light is not able to penetrate as far into the sample as much as it once was able to. These results allude to the fact that the skin samples are becoming less permeable to near infrared light as they continue to age.

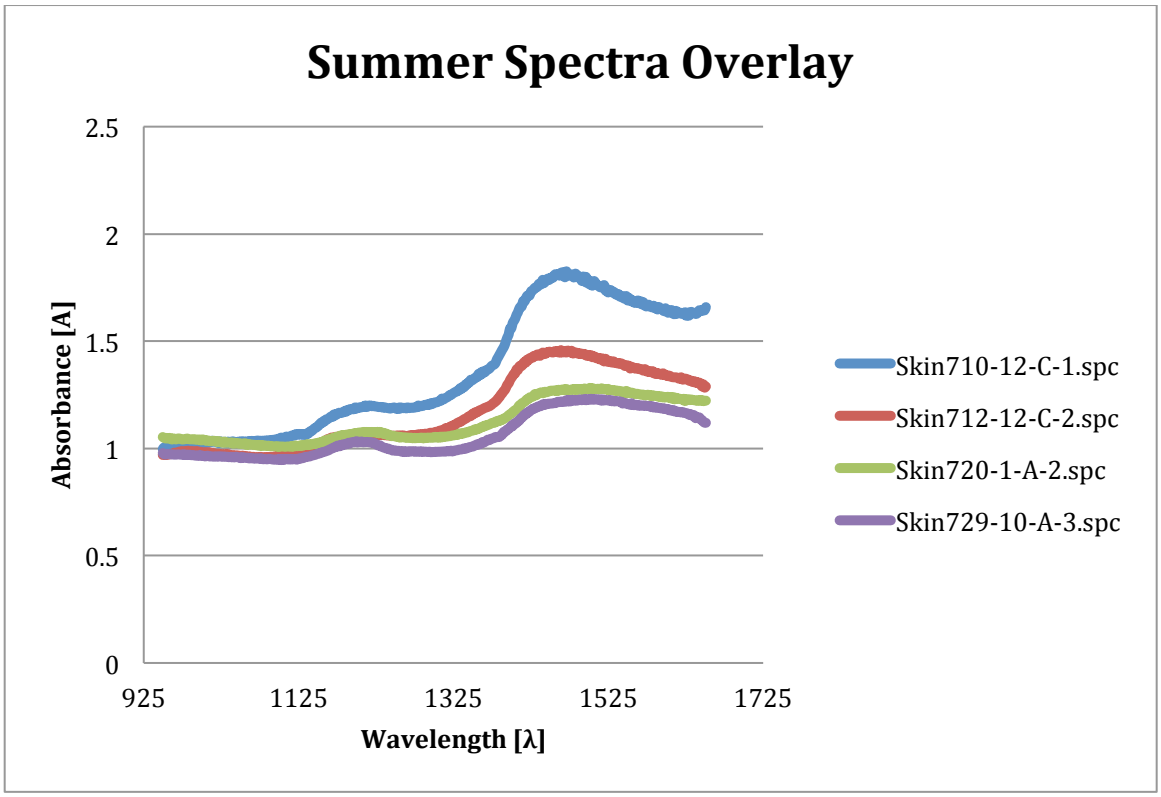

Figure 3. Four NIRS spectra from skin of one decomposing swine pastern exposed in July (Summer) comparing initial reading (blue), to successively ageing sample conditions (red, green and purple, respectively).

Figure 3 shows four individual spectra that were taken across the month of July (Summer). The four spectra were physically read on the 10th, 12th, 20th, and 29th. The gradual decrease in absorbance across the entire range of near-infrared wavelength helps to show the dermal sample's ongoing decline in absorbing this particular range of light.

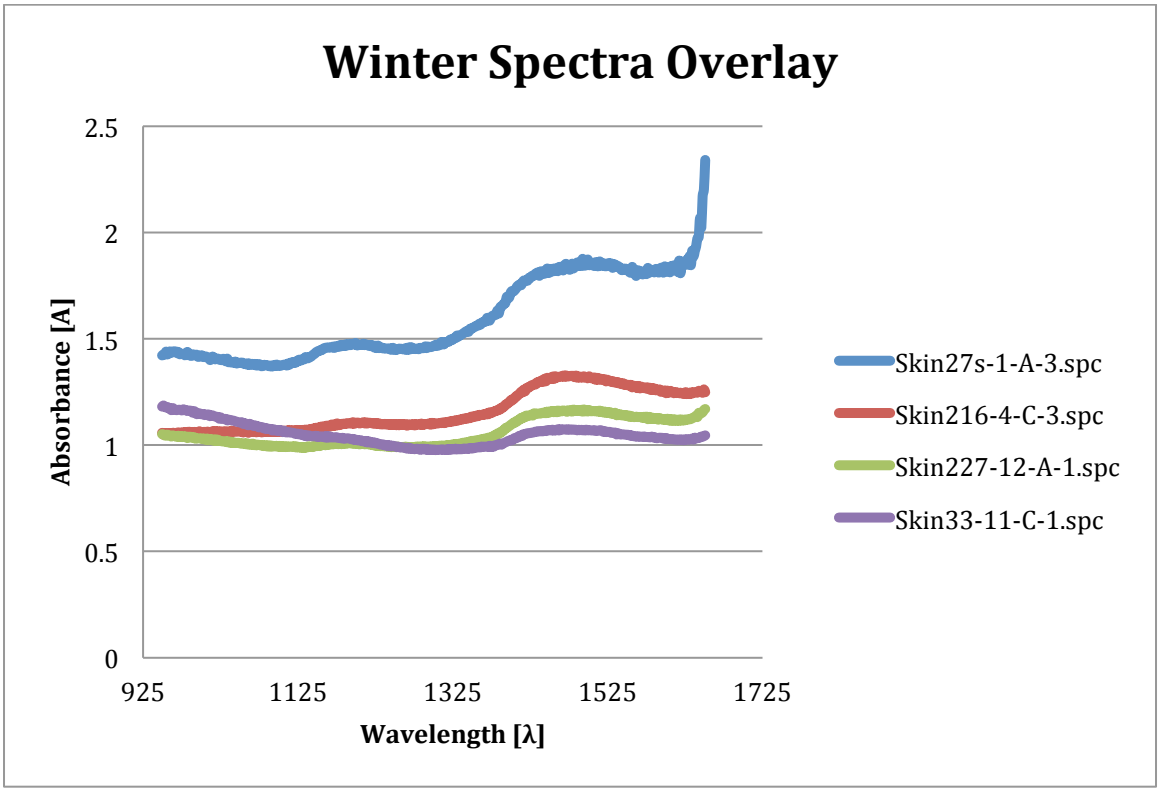

Figure 4. Four NIRS spectra from skin of one decomposing swine pastern exposed in February-March (Winter) comparing initial reading (blue), to successively ageing sample conditions (red, green and purple, respectively).

Figure 4 shows four individual spectra that were taken across the month of February and March (Winter). The four spectra were physically read on the 7th, 16th, and 27th of February as well as the 3rd of March. The gradual decrease in absorbance across the entire range of near-infrared wavelength helps to show the dermal sample's ongoing decline in absorbing this particular range of light, and confirms this trend of decreased absorbance when compared to that of the warm weather spectra.

CHAPTER IV

CONCLUSION

This research was meant to serve as the starting point, with data and results that other laboratories and research teams can use as a solid foundation and beginning for the application of this method. The entire purpose of this work was to serve as a proof of concept that NIRS can indeed be used to observe changes in decomposing tissue samples, and potentially provide direction for future studies associating NIRS technology with determinations of the post-mortem interval.

Spectra of swine dermal tissue were compiled and analyzed to estimate how well correlated the NIRS spectra were associated to sample age. Spectral data obtained from both human hair and swine hoof samples have yet to be processed, and have yet to be fully analyzed through the steps of our protocol. Our focus was limited to the linear regression analysis of the warm and cool exposure data from strictly dermal tissue. Initial linear regression analysis indicates a model with an R^2 of 0.8749, for our warm exposure spectral bank, provided the best fit of spectra to sample age. We also recorded an R^2 of 0.812 for our linear regression analysis of the cool exposure data. Visually (Figures 1 and 2) however, the rate of spectra change between readings appeared to slow with sample age indicating a bend in the relationship of spectra and age. I predict that the spectra will continue a progressive slower change with increasing sample age. Further, the application of non-linear regression may provide an even better fit of NIRS spectra to sample age. That NIRS spectral data can be associated with decomposition of skin tissue indicates potential for this technology to be applied to the estimation of post mortem intervals in death

scene investigations. Modeled results can now be used to test validity of post mortem intervals in future experiments.

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