Examining the Effects of Freeze/Thaw Cycles on Porcine Skin Using Spectral Analysis

Michelle Faggert; Namrata Gupta; Curtis Overton; Joseph Peller; Susan Trammell

UNC CHARLOTTE

CTI Charlotte Teachers Institute
Collaborative Teacher Education

Optical Spectroscopy for Cancer Detection

- Cancer is the second leading cause of death in the United States. In 2017, the American Cancer Society estimates that there will be 1,688,780 new cancer diagnoses and 600,920 cancer deaths in the US alone.[1]
- Currently, surgeons rely on imaging techniques such as MRI, CT and X-Ray to create preliminary surgical plans. While these imaging techniques help surgeons identify the locations of tumors, they cannot be used in real-time during surgery.
- During procedures, surgeon rely on visual observation and experience to determine the extent of cancerous tissue. Leaving behind cancerous tissue can result in high mortality rates as the result of recurrent tumors.
- A real-time diagnostic imaging tool that could identify cancerous region during surgery would result in better patient outcomes.
- Recent research has demonstrated that optical spectroscopy can be used to distinguish between healthy and diseased tissue and may become an important minimally invasive diagnostic tool for a range of diseases including breast cancer, colon cancer, cervical cancer, and Barrett's esophagus.
- Peller et al. are developing a new real-time imaging system for cancer detection.[2]
- Peller et al. used porcine skin samples in preliminary studies to determine the ability of the imaging system to detect changes in tissue properties.
- This study evaluates the effects of long-term sub-zero storage on the reflectance and autofluorescent spectra of the tissue to determine when and if degradation of the spectra becomes significant.

Experimental Setup: Spectroscopy of Porcine Skin Tissue

- A picture of the experimental setup is shown in Figure 1. Both reflectance and autofluorescent spectra of porcine skins samples (N=5) were obtained to evaluate the effect of freezing samples on the characteristics of the spectra.
- Porcine skin tissue samples of uniform thickness were cut into 2 cm x 2 cm squares.
- A 3 mW nitrogen laser (SRS, NL100) was used as the autofluorescence excitation source.
- The laser has a center wavelength of 337.1 nm, a 0.1 nm optical bandwidth, 170 μJ pulse energy, and a 10 Hz pulse rate. The beam size was approximately 0.5 cm x 1 cm resulting in an energy density of about 8.5 J/m^2 on the tissue.
- The laser was mounted parallel to the stage and the beam directed via a steering mirror onto the sample at approximately 40° from the normal of the sample.
- A 300 W Xenon lamp (Circon, MV-9086) was used as the source for reflectance spectroscopy and was mounted 42° from the vertical axis and aligned to illuminate the specimen stage.
- The light was collected by an Ocean Optics QE Pro Spectrometer (spectral range 350-750 nm, 1044 pixels in the linear array, and dispersion 0.335 nm/pixel).

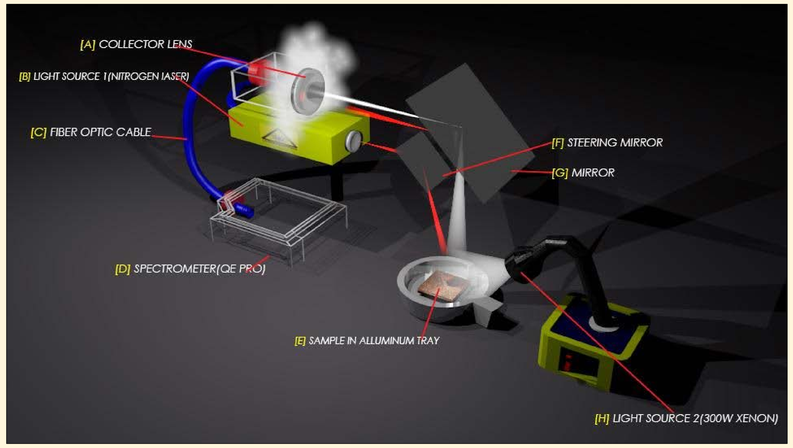

Figure 1: A schematic of the spectroscopy setup used in this setup. The sample stage (E) is illuminated by either the nitrogen laser (B) directed by a steering mirror(F) or from a xenon lamp (H). The reflected light or fluorescence from the tissue is reflected from the mirror(G) into the collector lens (A) and through an optical fiber(C) into a spectrometer. (D)

- Porcine skin samples were stored in saline solution at subzero temperatures. For each trial, one sample was thawed in warm water and placed on the specimen stage.
- The sample was illuminated with the Xenon source and the reflectance spectrum was recorded. The same sample was illuminated with the nitrogen laser and the autofluorescence spectrum was measured.
- The sample was then placed in saline solution and placed in the freezer to refreeze. Nine trials were completed.

Results and Analysis

- Three distinct features are evident in the reflectance spectrum (Figure 2). Feature 1 covers the wavelength range 400-450 nm, feature 2 is between 520-560 nm, and feature 3 is between 565-580 nm.
- The strength of each features was measured by summing the intensity over the wavelength range of the feature and then compared over freezing/thawing cycles to look for changes in the reflectance spectrum.
- The fluorescence spectrum exhibits one distinct peak, between 415-510 nm (Figure 2).
- The strength of this features was monitored over freezing/thawing cycles to examine the effect of freezing on the fluorescence spectrum of the tissue.

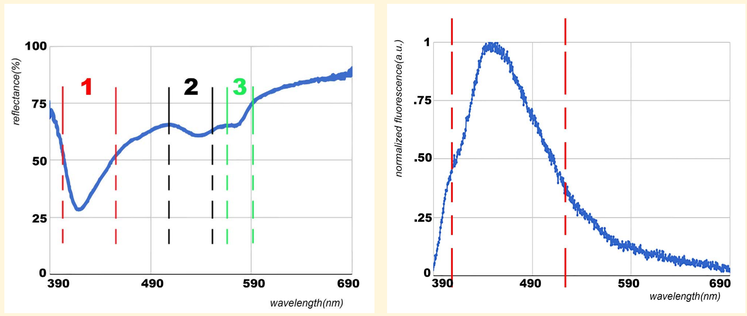

Figure 2: A representative spectra of the reflectance and fluorescence of porcine skin tissue. In the reflectance spectra 3 scattering features were identified and the single fluorescence peak was also identified. Each feature strength was examined over each freeze thaw cycle.

- Table 1 shows the average percent change with the standard deviation of the mean for the feature strengths observed in both the reflectance and fluorescence spectra in all samples (N=5) over the course of all freeze/thaw cycles (trials=9).
- The fluorescence spectrum does not change significantly over the nine trials. This suggests that the chemical composition of the skin sample has not changed.
- The strengths of the features seen in the reflectance spectra change significantly over the nine trials, with more freezing/thawing cycles resulting in more change.
- Figure 3 shows the percent change of features 2 and 3 (the results for feature 1 are similar) over all nine trials. After the fourth trial a significant difference in the reflectance spectrum is apparent. These changes in the reflectance spectrum are probably due to a changes in the morphology of the skin cells.

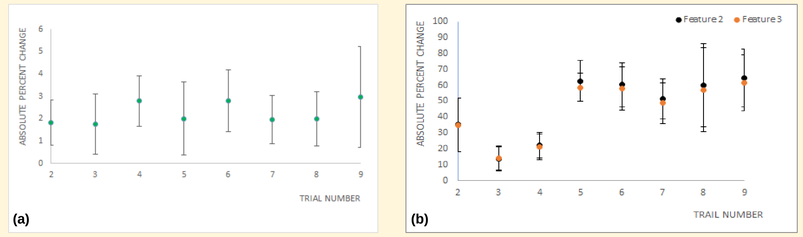

Figure 3:Graph of the average percent change of the fluorescence features (a) and figures 2 and 3 (b) over the trials. In the fluorescence spectra no significant change was observed over 9 trials. In the reflectance spectra there was a significant change in the strength of the features after trial 4 . In addition, the standard deviations at later trials probably corresponded to a degradation of the tissue structure.

Table 1:Average % change of feature strengths.

Trial Number	Average % change of Reflectance Feature 1	Average % change of Reflectance Feature 2	Average % change of Reflectance Feature 3	Average % change of Fluorescence Feature
2	41 ± 19	35 ± 17	35 ± 17	2 ± 1
3	16 ± 8	14 ± 7	14 ± 8	2 ± 1
4	35 ± 8	22 ± 8	21 ± 8	3 ± 1
5	96 ± 46	63 ± 13	59 ± 9	2 ± 2
6	82 ± 23	60 ± 14	58 ± 14	3 ± 1
7	77 ± 18	51 ± 12	49 ± 13	2 ± 1
8	91 ± 40	60 ± 26	57 ± 26	2 ± 1
9	90 ± 39	65 ± 18	61 ± 18	3 ± 2

Discussion and Future Work

- The study indicates that there is a significant change in the reflectance spectrum after the fourth trial. A significant change does not occur in the fluorescence spectrum.
- Future research might entail examining the mechanisms causing changes in the reflectance spectrum by observing skin samples under a microscope.
- Cycles of freezing and thawing should be continued until there is a noticeable difference in the normalized fluorescence spectrum

References:

[1] Siegel, R. L., Miller, K. D. and Jemal, A. (2017), Cancer statistics, 2017. CA: A Cancer Journal for Clinicians, 67: 7–30. doi:10.3322/caac.21387
[2] Peller, J., Thompson, K. J., Siddiqui, J., Martinie, J., Iannitti, D. A., & Trammell, S. R. (2017, February). Hyperspectral imaging based on compressive sensing to determine cancer margins in human pancreatic tissue ex vivo. In Proc. of SPIE Vol (Vol. 10060, pp. 100600J-1).
[3] Z. Volynskaya, A. S. Haka, K. L. Bechtel, M. Fitzmaurice, R. Shenk, N. Wang, J. Nazemi, R. R. Dasari, and M. S. Feld, "Diagnosing breast cancer using diffuse reflectance spectroscopy and intrinsic fluorescence spectroscopy," J. Biomed. Opt. 13(2), 024012 (2008).
[4] Chandra M, Scheiman J, Simeone D, McKenna B, Purdy J, Mycek M, "Spectral areas and ratios classifier algorithm for pancreatic tissue classification using optical spectroscopy," J. Biomed. Opt, 0001;15(1):010514-010514-3
[5] G. Zonios, L. T. Perelman, V. Backman, R. Manoharan, M. Fitzmaurice, J. V Dam, and M. S. Feld, "Diffuse reflectance spectroscopy of human adenomatous colon polyps in vivo," Appl. Opt. 38(31), 6628–6637 (1999)
[6] S. K. Chang, N. Marin, M. Follen, and R. Richards-Kortum, "Model-based analysis of clinical fluorescence spectroscopy for in vivo detection of cervical intraepithelial dysplasia," J. Biomed. Opt. 11(2), 024008 (2006)
[7] Georgakoudi, Irene et al. "Fluorescence, reflectance, and light-scattering spectroscopy for evaluating dysplasia in patients with Barrett's esophagus," Gastroenterology , Volume 120 , Issue 7 , 1620 – 1629