# FTIR spectroscopy: an optical method to study wound healing process

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**Abstract:** In this study, we investigated the ability of Fourier transform infrared spectroscopy to discriminate healthy tissue and thermal injury, aiming the development of an optical method to evaluate the wound healing process. 
**OCIS codes:** (170.0170) Medical optics and biotechnology; (170.6510) Spectroscopy, tissue diagnostics.

## 1. Introduction

Wound healing is a complex process in which the skin and the surrounding tissue are repaired after injury. The healing process is characterized by four phases: Inflammation, transition, proliferative and maturative [1]. The biochemical events occurring in each phase are still not fully understood, therefore systematic comparative tests are necessary. Usually, the gold standard method to study the molecular biology of wound healing is based on immunohistochemical analysis, which is laborious, time-consuming and require multiple assays [2]. Considering that Fourier transform infrared spectroscopy (FTIR) has been well succeeded analyzing biological samples, the present study aims to demonstrate the ability of FTIR to discriminate healthy and burned skin in the transition stage.

## 2. Material and Methods

*Animal Experiment and Sample preparation*

After approval of Ethics Committee for Animal Research from Nuclear and Energy Research Institute (CEUA IPEN 165/15), burn injuries were induced in the back of Wistar rats using water vapor at 90 ºC. In order to evaluate the healing process, tissue specimens were extracted after 7 days of burn injurie protocol and cryopreserved [3]. Tissue sections of 5 µm thickness were placed in MirrIR low-E-coated slides (Kevley Technologies, Chesterland, OH).

*FTIR Spectroscopy*

Spectral data were collected using a FTIR system (Thermo Nicolet 6700, Waltham, MA) operating with an Attenuated Total Reflectance (ATR) accessory as sampling mode (Smart Orbit, Thermo cientific, Waltham, MA). Measurements were performed in MID-infrared region (4000–400 $cm^{-1}$) with spectral resolution of 4 $cm^{-1}$ and 150 scans per spectrum.

*Chemometric Analysis*

Fingerprint region (900-1800 $cm^{-1}$) of spectra were offset-corrected and normalized by amide II band area. Second derivative was calculated in order to assess the overlapped sub-bands and submitted to Savitzky-Golay filter with a polynomial of second order in an eleven-points window [4]. Principal Component Analysis (PCA) was used to evaluate the biochemical changes on spectral data. All chemometric methods were carried out on MATLAB® R2015a (MathWorks, Natick, MA).

## 3. Results and Discussion

Figures 1.A and B show the averaged spectrum of the fingerprint region and their second derivative.

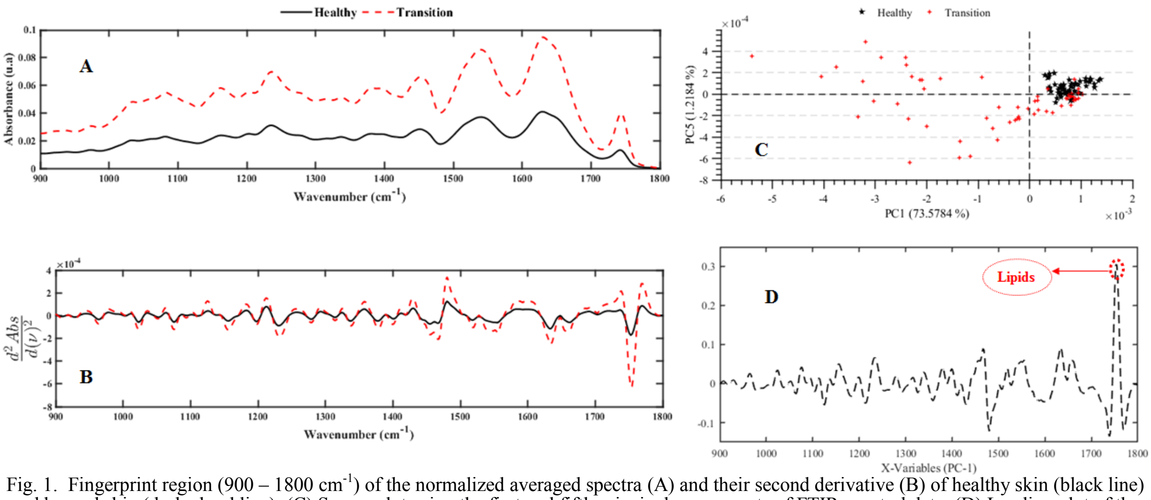


Fig. 1. Fingerprint region (900 – 1800 $cm^{-1}$) of the normalized averaged spectra (A) and their second derivative (B) of healthy skin (black line) and burned skin (dashed red line); (C) Scores plot using the first and fifth principal components of FTIR spectral data; (D) Loading plot of the first principal component.

Figures 1.C depicts the score plot of the first and fifth principal components obtained for burn wound tissue spectra pairwise compared to healthy skin. Satisfactory data discrimination was obtained along the first principal component, in which most scores from burn wound tissue lie on negative values of PC-1 axis and scores from healthy skin are grouped in the positive values. Positive loading was evidenced in the band peaking at 1750 $cm^{-1}$, which is attributed to C-O stretching of triglycerides and cholesterol esters [5], therefore suggesting an increase in the lipid content [7–8] in the thermal injured tissue. These findings are associated to the oxidative stress induced by free oxygen radicals and may be used as a spectral biomarker to better understand the biochemical events during the wound healing process.

## 4. Acknowledgment

This work was supported by FAPESP CEPID 05/51689-2, CAPES/PROCAD 88881.068505/2014-01, CNPq/ INCT 465763/2014-6, 141946/2018-0 and PQ 309902/2017-7.

## 5. References

[1] N. X. Landen, D. Li, and M. Stahle, "Transition from inflammation to proliferation: a critical step during wound healing,": CMLS **73**, 3861-3885 (2016).

[2] A. Salibian, A. T. D. Rosario, L. A. M. Severo, L. Nguyen, D. A. Banyard, J. D. Toranto, G. R. D. Evans, and A. D. Widgerow, "Current concepts on burn wound conversion-A review of recent advances in understanding the secondary progressions of burns," Burns : journal of the International Society for Burn Injuries **42**, 1025-1035 (2016).

[3] M. J. Baker, J. Trevisan, P. Bassan, R. Bhargava, H. J. Butler, K. M. Dorling, P. R. Fielden, S. W. Fogarty, N. J. Fullwood, K. A. Heys, C. Hughes, P. Lasch, P. L. Martin-Hirsch, B. Obinaju, G. D. Sockalingum, J. Sule-Suso, R. J. Strong, M. J. Walsh, B. R. Wood, P. Gardner, and F. L. Martin, "Using Fourier transform IR spectroscopy to analyze biological materials," Nature protocols **9**, 1771-1791 (2014).

[4] L. C. Lee, C.-Y. Liong, and A. A. Jemain, "A contemporary review on Data Preprocessing (DP) practice strategy in ATR-FTIR spectrum," Chemometrics and Intelligent Laboratory Systems **163**, 64-75 (2017).

[5] C. Matthäus, B. Bird, M. Miljković, T. Chernenko, M. Romeo, and M. Diem, "Chapter 10 Infrared and Raman Microscopy in Cell Biology," **89**, 275-308 (2008).

[6] Z. Movasaghi, S. Rehman, and D. I. ur Rehman, "Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues," Applied Spectroscopy Reviews **43**, 134-179 (2008).

[7] Z K. L. Andrew Chan and S. G. Kazarian, "Attenuated total reflection Fourier-transform infrared (ATR-FTIR) imaging of tissues and live cells," Chemical Society reviews **45**, 1850-1864 (2016).

[8] G. C. Gurtner, S. Werner, Y. Barrandon, and M. T. Longaker, "Wound repair and regeneration," Nature **453**, 314-321 (2008).