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A Classification Model for Non-alcoholic Steatohepatitis (NASH) Using Confocal Raman Micro-spectroscopy

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Abstract: We combined Raman micro-spectroscopy and machine learning techniques to develop a classification model based on a well-established non-alcoholic steatohepatitis (NASH) mouse model, using spectrum pre-processing, biochemical component analysis (BCA) and logistic regression.
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Background: Clinically, the histopathological evaluation of liver biopsies is currently the gold standard for non-alcoholic steatohepatitis (NASH) diagnosis [1]. However, significant variation exists amongst pathologists in the definition of NASH and an unequivocal agreement has not been reached. In sum, whether for diagnosing NASH or for NAFLD staging, the traditional histological approach is fundamentally semi-quantitative, observer-dependent, and includes only a very limited set of pathological features [2]. Besides being a label-free approach that enables multiplexing, Raman micro-spectroscopy provides a biochemical map of the tissue of interest that potentially enables the identification of spatial-temporal changes in tissue composition [3]. Thus, to address the disease progression and spatiotemporal information, we have created a fully quantitative and objective approach to NASH detection.

Methods: We combined spontaneous Raman micro-spectroscopy and machine learning techniques to identify spectral signatures that are specific to NASH from the liver tissue provided by 42 STAM^{TM} mice that were administrated with Streptozotocin (STZ), fed with high fat diet and sacrificed at 6 time points, by using spectrum pre-processing, biochemical component analysis (BCA) and logistic regression.

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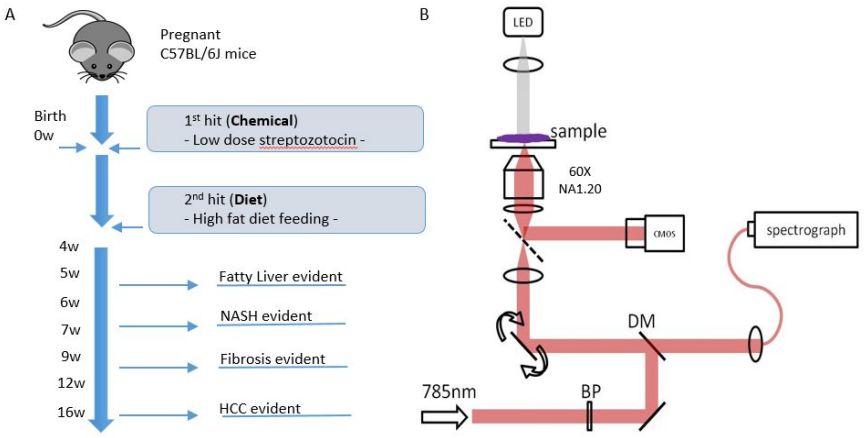


Fig. 1. A) Figure illustration of STAM mice model. The model was created by using both the effect of chemical toxic STZ (0 week) and high fat dietary (4 weeks) on non-genetic C57BL/6 mice with measurements conducted on the 5 different time points. A total of 42 cryo-preserved tissues were harvested from the mice. B) Schematic illustration of Raman micro-spectroscope set-up. Excitation laser was a continuous wave (CW) tunable Ti: Sapphire laser (wavelength set at 785nm) with a frequency-doubles Nd: YAG laser (wavelength set at 532nm) used as the pump source. The collimated beam passed through a bandpass filter (BP LL01-785-12.5, Semrock), a dichroic mirror (DM LPD01-785RU-25, Semrock), an objective lens (60X, NA=1.2), the dual-axis galvanometer mirrors and adjusted by a telescope before being focused on the tissue specimen. The inelastic scattering Raman signal was then delivered to the spectrograph by using the Raman grating which has the spectra shift coverage of -34 to 1894 cm^{-1} and the spectra resolution of 2.0 cm^{-1}/pixel. Spectra are captured by a liquid Nitrogen cooled charge-coupled device (CCD) and the bright field images were captured using an intensity controlled white light-emitting diode (LED) and the complementary metal-oxide semiconductor (CMOS) camera.

Results: We showed that Raman micro-spectroscopy can detect macro-steatosis with variation in both its size and distribution, as a characteristic feature of NASH. Raman reconstructed images were also able to reveal the spatiotemporal information during the disease progression. By employing a selected pool of biochemical components to represent the liver constituents, we also identified biochemical changes specific to NASH and show that the classification model is capable of accurately detecting NASH (Area Under Curve (AUC) = 0.87) in mice.

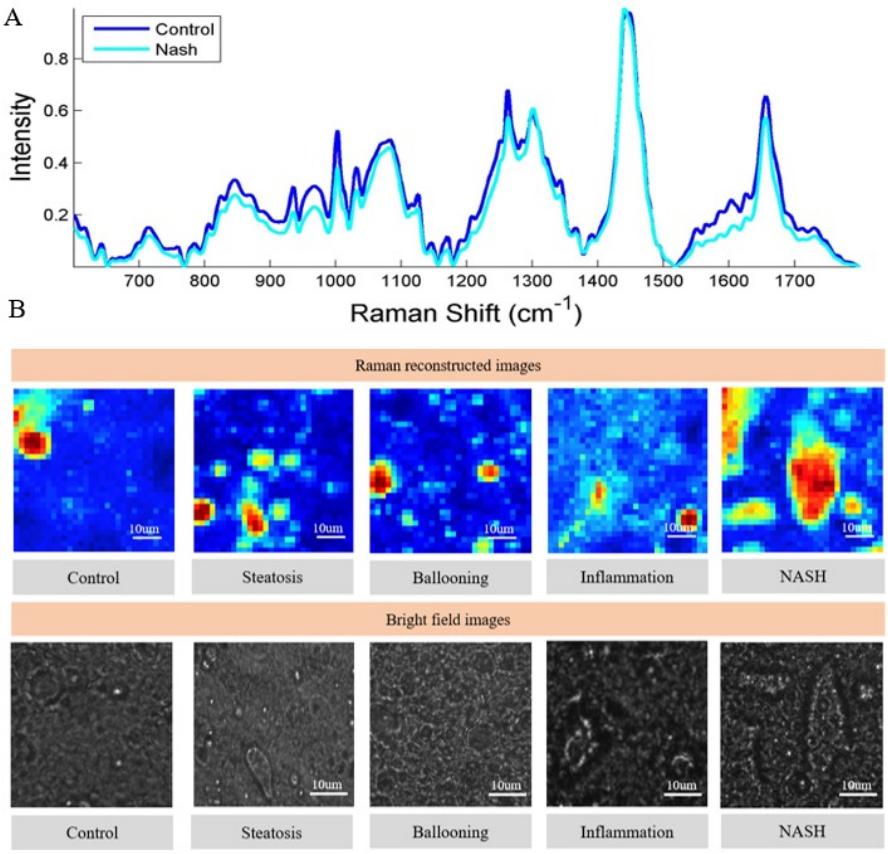
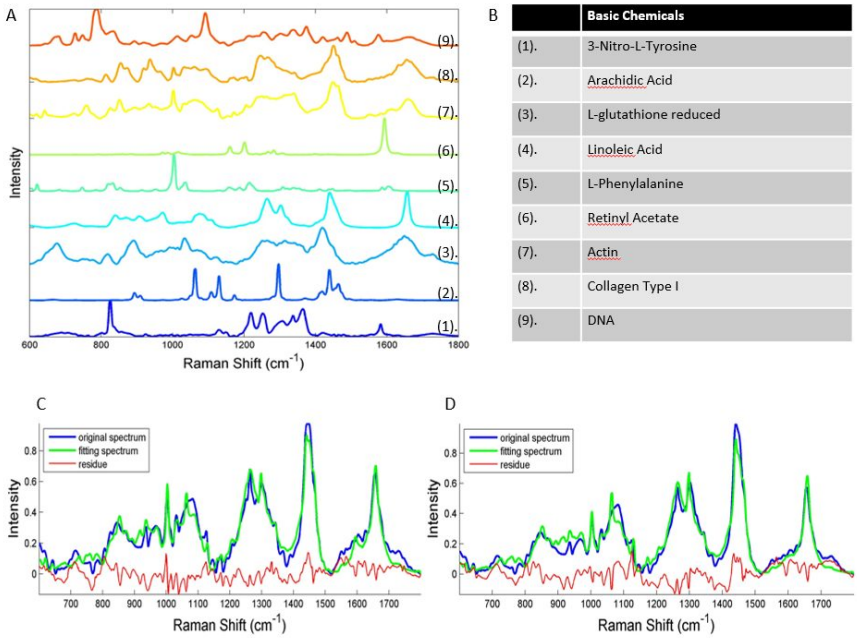


Fig. 2. A) The averaged Raman spectrum of control and Non-alcoholic steatohepatitis (NASH) groups across 871 Raman shifts. B) Raman reconstructed image using the intensity at the Raman shift of 1655 cm^{-1} and respective bright field image from representative control, steatosis, ballooning, inflammation and NASH group to reveal the spatiotemporal information.



	Basic Chemicals
(1).	3-Nitro-L-Tyrosine
(2).	Arachidic Acid
(3).	L-glutathione reduced
(4).	Linoleic Acid
(5).	L-Phenylalanine
(6).	Retinyl Acetate
(7).	Actin
(8).	Collagen Type I
(9).	DNA

Fig. 3. A) The Raman spectra of the selected nine compounds selected for the Raman signal decomposition. B) The name of the nine compounds. C) and D) The spectrum fitting result of the control and Non-alcoholic steatohepatitis (NASH) groups. The blue line represents the

original spectrum and the green one represents the reconstructed spectrum using the nine selected compounds after spectrum decomposition while the red line gives the residual.

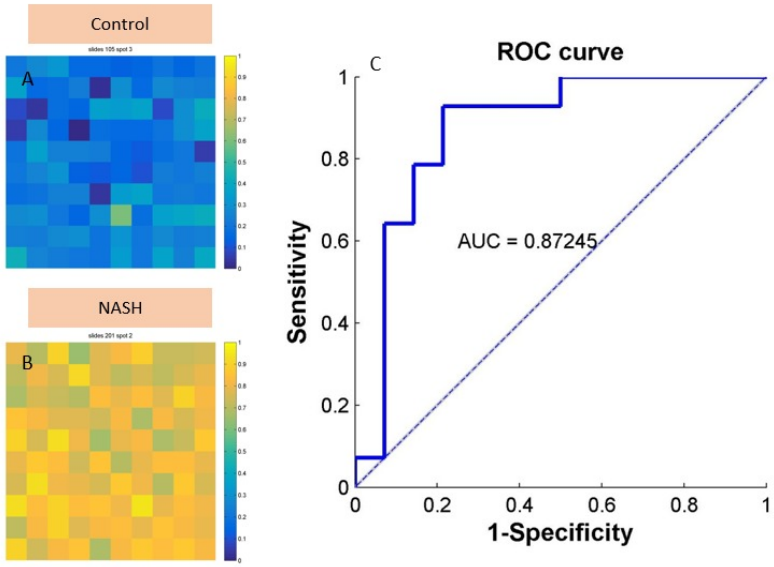


Fig. 4. A) and B) The diagnostic plot for a true negative and true positive sample where the pixels' intensity represents the probability of being diagnosed as normal or NASH. C) The receiver operating characteristics curve (ROC) analysis of the classification model.

Conclusion: In this study, we demonstrate that Raman micro-spectroscopy can similarly be used for the detection of NAFLD/NASH through quantification of the biochemical and biological changes at the cell and tissue level with spatiotemporal resolution. The unique biochemical fingerprint generated in this study may serve as a useful criterion to be leveraged for further validation in clinical samples.

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