Supplementary Appendix

a. What is RS

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorph, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material. Raman is a light scattering technique, whereby a molecule scatters incident light from a high intensity laser light source. Most of the scattered light is at the same wavelength (or color) as the laser source and does not provide useful information – this is called the Rayleigh Scatter. However, a small amount of light (typically 0.0000001%) is scattered at different wavelengths (or colors), which depend on the chemical structure of the analyte – this is called Raman Scatter.

A Raman spectrum features a number of peaks, showing the intensity and wavelength position of the Raman scattered light. Each peak corresponds to a specific molecular bond vibration, including individual bonds such as C-C, C=C, N-O, C-H etc., and groups of bonds such as benzene ring breathing mode, polymer chain vibrations, lattice modes, etc.

b. What is SERS

Raman signals are inherently weak, especially when using visible light excitation and so a low number of scattered photons are available for detection. SERS maintains the advantages of normal RS but enhances the signal intensity of Raman scattering for

sensitive detection, while suppresses fluorescence emissions at the same time. SERS uses nanoscale roughened metal surfaces typically made of gold (Au) or silver (Ag). Laser excitation of these roughened metal nanostructures resonantly drives the surface charges creating a highly localized (plasmonic) light field. When a molecule is absorbed or lies close to the enhanced field at the surface, a large enhancement in the Raman signal can be observed. Raman signals several orders of magnitude greater than normal Raman scattering are common, thereby making it possible to detect low concentrations (10^{-11}) without the need for fluorescent labeling. The Raman signal can be amplified further when the roughened metal surface is used in combination with laser light that is matched to the absorption maxima of the molecule.

c. How SERS going to detect the changes relevant to NAFLD spectrum from serum

The changing levels of a variety of serum biomarkers may reflect NAFLD severity. These biomarkers range from simple biochemical (serum aminotransferases, bilirubin and ferritin levels), metabolic (hemoglobin A1c [HbA1c], insulin and HOMA-insulin resistance score) and lipid (triglycerides, cholesterol) parameters to complex biomarkers reflecting specific molecular mechanisms underlying the pathogenesis and progression of NAFLD, including inflammation, oxidative stress, apoptosis, and glucose and lipid metabolism. As the pathogenesis of NASH is complex and likely involves multiple biological abnormalities, it is unlikely that a single biomarker could accurately discriminate between simple steatosis (NAFL) and NASH. SERS typically

features a number of peaks, and each peak corresponds to a specific molecular bond vibration, including individual bonds such as C-C, C=C, N-O, C-H etc. SERS can provide label-free fingerprinting type information on vibrational and rotational modes of chemical structures including metabolites, glucose, proteins, lipids, and nucleic acids. The distinctive SERS spectral features and intensity differences for NASH and NAFL patient groups can reflect molecular and cellular changes associated with progression from NAFL to NASH.

d. How the SERS images are acquired

1.5 μL of silver nanoparticles (AgNPs) were added onto the pre-cleaned aluminum plate, immediately the serum with the same volume was then gently added and mixed with AgNPs, and dried at room temperature. Prior to determination, the system was calibrated with the Raman peak of single crystalline silicon at 520 cm^{-1}. SERS spectra were recorded with a confocal Raman micro-spectrometer (Renishaw Invia, UK) in the range of 400-1800 cm^{-1} under a 785nm diode laser excitation. The spectra were collected in backscattering geometry using a microscope equipped with a Leica 20× objective with a spectral resolution of 2cm^{-1}; the detection of Raman signal was carried out with a Peltier cooled charge-coupled device (CCD) camera. The software package WIRE 2.0 (Renishaw) was employed for spectral acquisition and analysis.