Aug 08, 2019

UC Davis - Lipoprotein analysis by LTRS V.2

Thomas Huser[1]

[1]University of California, Davis

1 Works for me dx.doi.org/10.17504/protocols.io.56yg9fw

Mouse Metabolic Phenotyping Centers
Tech. support email: info@mmpc.org

Lili Liang

ABSTRACT

Summary:

Raman spectroscopy analyzes non-elastically scattered light from chemical bonds (Fig. 1). Photons from a laser source are scattered by interaction with molecular bond vibrations and either deposit energy into a particular bond or receive additional energy from an already excited bond vibration. Different molecular bonds have different vibrational frequencies and lead to specific peaks in the Raman spectrum. For instance, a C-C bond can be easily distinguished from a C=C bond, allowing us to distinguish saturated from unsaturated lipids. All biomacromolecules, including DNA, RNA, proteins, and lipids, have characteristic Raman modes, which enable the nondestructive determination of their chemical composition and structure. Raman spectroscopy provides complementary information to mass spectrometry or magnetic resonance imaging (MRI) without destroying cells in the process of the analysis, with high spatial resolution akin to confocal optical microscopy, and without the need for contrast agents - enabling live imaging at the cellular level.
In our LTRS system we have further increased the sensitivity of Raman spectroscopy at the cellular and sub cellular level by combining laser trapping and Raman spectroscopy. Here, a tightly focused laser beam, such as the one obtained by sending the beam through a high resolution immersion oil microscope objective, forms an optical laser trap which captures cells or lipoproteins and confines them to the laser focus until the laser is turned off or another particle collides with the one captured.

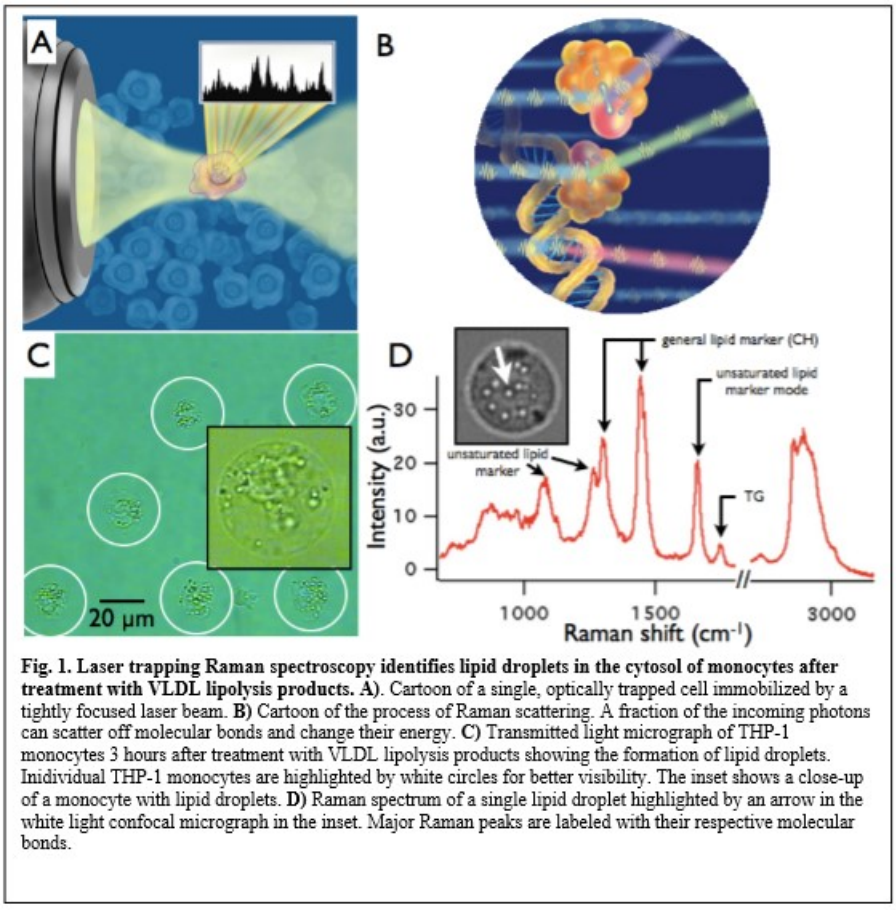

Fig. 1. Laser trapping Raman spectroscopy identifies lipid droplets in the cytosol of monocytes after treatment with VLDL lipolysis products. A). Cartoon of a single, optically trapped cell immobilized by a tightly focused laser beam. **B)** Cartoon of the process of Raman scattering. A fraction of the incoming photons can scatter off molecular bonds and change their energy. **C)** Transmitted light micrograph of THP-1 monocytes 3 hours after treatment with VLDL lipolysis products showing the formation of lipid droplets. Inidividual THP-1 monocytes are highlighted by white circles for better visibility. The inset shows a close-up of a monocyte with lipid droplets. **D)** Raman spectrum of a single lipid droplet highlighted by an arrow in the white light confocal micrograph in the inset. Major Raman peaks are labeled with their respective molecular bonds.

Modified from: Chan JW, et.al. Anal. Chem. 2005 Sep 15;77(18):5870-76.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=130&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
DPBS	14190	Invitrogen - Thermo Fisher
formaldehyde	F79	Fisher Scientific
ultracentrifuge tubes		Beckman Coulter
Triglyceride Concentration kit		Sigma Diagnostics
#1 or 1.5 coverglass		Fisher Scientific

MATERIALS TEXT

Reagent Preparation:

Reagent 1: 4% paraformaldehyde
Formaldehyde (Fisher) is diluted to 4% in DPBS (Invitrogen)

Note:
Fisher Scientific, RRID:SCR_008452
Sigma-Aldrich RRID:SCR_008988
Beckman Coulter, RRID:SCR_008940

SAFETY WARNINGS

WARNING:
Formalin is, toxic, flammable and considered a carcinogen. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

1 Preparation of TGRL samples

1. Blood is obtained from volunteers by venipuncture and collected in 10 ml vacutainer tubes filled with streptokinase (150 units/ml blood).

2. Whole blood is then spun at 4ºC for 10 minutes at 1750g, to remove cellular particulate.

3. Plasma is transferred to ultracentrifuge tubes (Beckman-Coulter) and centrifuged at 285,000g at 4ºC for 4 hours to isolate TGRL. This procedure removes all higher density lipoprotein particles (LDL, HDL, and IDL) with a diameter of less then 40 nm.

4. After ultracentrifugation, TGRL are removed and the concentration is measured using an Infinity *Triglyceride Concentration kit* (Sigma Diagnostics).

5. The final samples containing only chylomicrons, VLDLs and their remnants are stored on dry ice and transported to the Oak Park Research Building in Sacramento, CA.

6. The plasma is diluted 1:100 in PBS buffer to separate and isolate individual TGRL in buffer solution.

7. Analysis of the TGRL by LTRS is typically conducted no later than 48 hours after extraction.

2 Other Sample Types and Preparation:

Similarly, lipid droplets inside cells (fixed or living) can be probed by directing the laser focus at individual lipid droplets inside cells.
1. Cells in culture
a. Live cells vs fixed- either live or fixed cells can be analysed, live cells will be switched from culture media to PBS prior to LTRS, fixed are fixed in 2 or 4% formaldehyde and switched to PBS prior to LTRS
b. Adherent cells- For extremely flat cells (endothelial cells, e.g.), we also use different substrates (special cut MgF2 or quartz glass windows)

2. Tissue
a. Tissue type- any tissue type can be subjected to LTRS- fresh, frozen, fixed in formaldehyde.
i. a glass coverslip (#1 or #1.5) will be placed on the tissue in order to make contact with the microscope lens
b. Tissue thickness- we can only measure up to ~ 100 µm deep

3 Acquisition of Raman spectra of single optically trapped Lipoprotein

1. The LTRS system consists of a 785 nm CW laser beam, spectrally filtered with a 785 nm bandpass filter and delivered into the upper side port of an Olympus IX71 inverted optical microscope.

2. A longpass dichroic reflector directs the beam through a 100X, 1.4 NA oil immersion, or 60x, 1.2 NA water immersion objective lens (Olympus), resulting in a diffraction limited spot of roughly 0.5 µm diameter with 32 mW laser power. The tight focus creates an optical trap that immobilizes single lipoprotein particles drifting in the plasma solution (**Figure 2**).

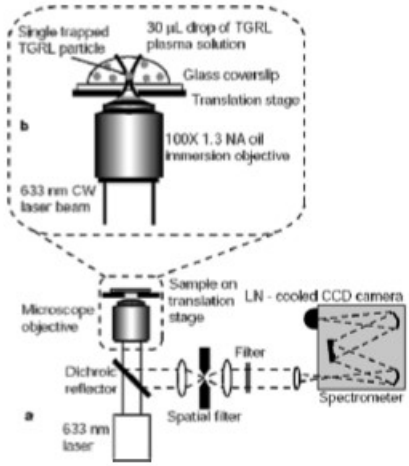

Figure 2. (a) Confocal spectroscopy system for the detection of the Raman spectra of single lipoprotein particles. (b) Optical trapping of a single lipoprotein particle in plasma solution or cell and simultaneous spectral acquisition using a single laser beam focused through a high numerical aperture objective.

3. White light illumination in transmission is used to obtain images captured on a CCD video camera.

4. Epi-detection of the Raman signals of the trapped particle is achieved using the same objective and a 100 µm pinhole in a confocal arrangement. The signals are filtered using a steep edge 785 nm long pass filter for suppression of residual laser light, directed into a spectrometer equipped with a 600 lines/mm grating, blazed at 800 nm, and focused onto a thermoelectrically cooled CCD camera (1340 x 100 pixels).

5. Optical trapping of single lipoprotein particles is accomplished by using the translation stage to move the particles to a close proximity of the laser focus, at which point the particle will be drawn into the focus and then trapped. A typical acquisition time of 60 seconds is sufficient to acquire a Raman spectrum of typically better than 10:1 signal-to-noise ratio with clearly defined Raman bands. Spectra are calibrated to a toluene standard and background correction is performed on each spectrum by subtraction of a 3rd order polynomial baseline fit. Each spectrum is normalized to the intensity of the 1440 cm^{-1} peak because fluctuations due to changes in protein concentration or conformation are relatively weak.

6. The differentiation of the differently sized lipoprotein particles (large vs. small) is based on their visibility in the optical microscope by comparison to polystyrene beads of well-known size.