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Compound Raman Microscopy for High-speed Vibrational Imaging and Spectral Analysis of Lipid Bodies

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Obesity is an established risk factor for type II diabetes, hypertension, strokes, many types of cancer, atherosclerosis, and other diseases [1]. A central goal of obesity studies is to understand how cells store excess energy in the form of cytoplasmic lipid droplets (LDs) [2]. Nonetheless, significant details on the biology of LDs are still lacking [3]. Currently, it is not clearly understood how different types of phospholipids or fatty acids contribute to the formation of LDs.

Until recently, the studies of lipid-droplet biology have been relying on non-specific, invasive, or population measurements. Recent advances in vibrational imaging are opening up exciting opportunities for dynamic, non-invasive, and compositional analysis of single LDs. However, the multiplexed techniques such as confocal Raman microscopy or multiplex CARS (M-CARS)[4, 5] lack speed (order of milliseconds to seconds per pixel) which restricts their use to relatively static samples. Single-frequency CARS microscopy[6] on the other hand permits high image acquisition speeds on the order of few μs/pixel but lacks the spectral information.

We demonstrate herein a compound Raman microscope that implements high-speed coherent Raman imaging of a biological sample and confocal Raman spectral analysis at points of interest using a ps laser source. With the capability of vibrational imaging and spectral analysis within a few seconds, the compound Raman microscope is applied to analyze the LDs in cultured Chinese ovary hamster (CHO) and 3T3-L1 cells, preadipocytes and mature adipocytes of explanted murine visceral adipose tissues, and subcutaneous adipocytes and sebaceous glands in a living BALB/c mouse (Fig. 1). Important information about lipid bodies including their abundance, size, morphology, degree of carbon chain unsaturation, and lipid-packing density can be accessed within a few seconds. Moreover, the compound Raman microscope allows tracking the cellular uptake of a specific fatty acid and its abundance in nascent cytoplasmic lipid droplets. The high-speed vibrational imaging and spectral analysis capability renders compound Raman microscopy an indispensible analytical tool to the studies of lipid-droplet biology.

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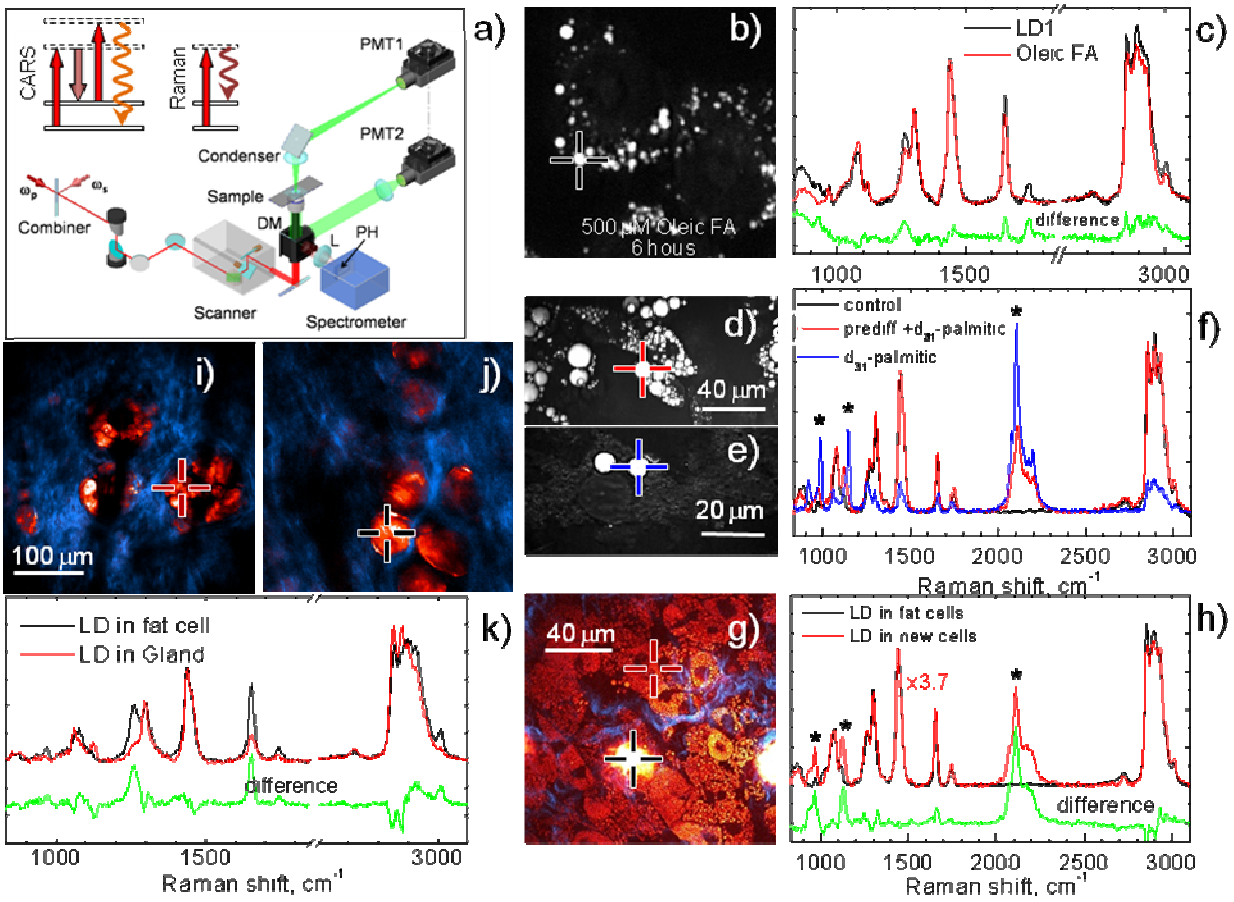

Fig. 1. Layout and performance of the compound Raman microscope. (**a**) Optical layout. ω_p and ω_s are pump and Stokes laser beams, DM is an exchangeable dichroic mirror, L is a 100 mm f.l. lens, PH is a 100-μm pinhole. PMT1 and PMT2 are photomultiplier tubes for forward(F) and backward (epi) detection. **(b)**, F-CARS image of CHO cells incubated for 6 h in medium contained 500 μM of oleic FA, **(d)** F-CARS image of 3T3-L1 cells differentiated for 2 weeks and incubated for 4 days in medium contained 50 μM of d_{31}-palmitic FA, **(e)** F-CARS image of 3T3-L1 cells incubated for 4 days in medium contained 50 μM of d_{31}-palmitic FA, **(g)** z-stacked epi-CARS image of mouse visceral fat (VF) (image is combined from E-CARS image (red) and SHG (light blue)), **(i-J)** epi-CARS images of overlapped CARS (red) and SHG (light blue) of *in vivo* mouse skin at two different depths.**(c)**, **(f)**, **(h)**, and **(k)** are corresponding Raman spectra obtained at the positions indicated by crosses in panels (b), (d), (e), (g), (i), and (k). Each CARS image is acquired at the speed of 2 μs/pixel. The CARS images and Raman spectra were obtained using a 60x IR water immerse objective (Olympus) and 10 mW of pump and 15 mW of Stokes at the sample. The acquisition times for Raman spectra are from 4 to 20 s.

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