A new way to look at fat

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Stimulated Raman scattering (SRS) microscopy is used to directly visualize lipids in cells and model organisms, and facilitates screening for genes involved in fat storage.

The World Health Organization has referred to obesity as "the most blatantly visible, yet most neglected public health problem"[1]. To better understand the biology of this disease, model systems are used to uncover the mechanisms that mediate and regulate fat metabolism. However, in model organisms or at the cellular level, fat is much less obvious to see or quantify. Most current microscopy-based methods have limitations in specificity, and biochemical analyses are cumbersome to perform and lack spatial information. To overcome this hurdle, Wang *et al.*[2] have adopted stimulated Raman scattering (SRS) microscopy to directly visualize and quantify cellular lipids by measuring the energy absorption of CH_2 vibrational stretching in aliphatic fatty acid chains (**Fig. 1**).

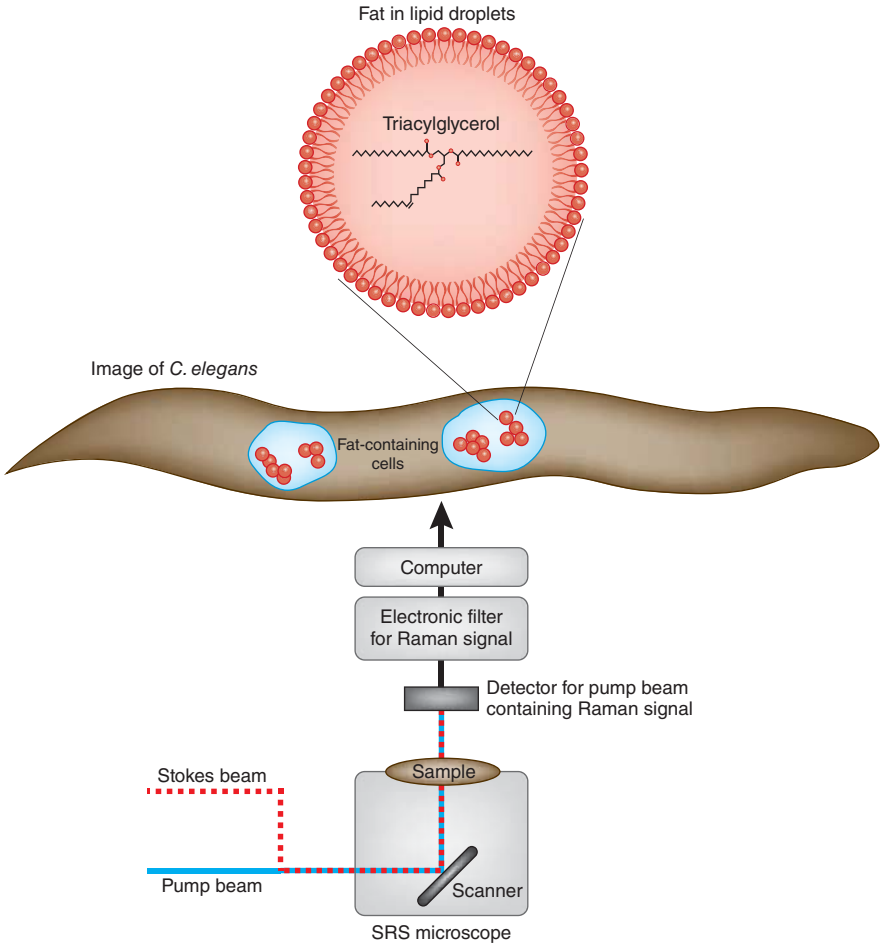

Figure 1 | SRS microscopy detects the scattered light from a pulsed Stokes and a pump beam tuned to a specific Raman shift, for example, characteristic of triacylglycerols in lipid droplets of *C. elegans*.

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SRS microscopy is thus a tool for analyzing fat depots in genetic model systems.

SRS microscopy is a recent and exciting development in the Raman spectroscopy field. In general, Raman spectroscopy is used to measure inelastic scattering that occurs when photons interact with matter. When a laser beam illuminates a sample, some of the photons transfer parts of their energy into vibrational modes of the sample's molecules in a process called Stokes scattering. Detection of the resultant spectrum shows peaks at shifted wavelengths (Raman shifts) that reflect these vibrational energy levels of the sample. Different molecules yield characteristic Raman fingerprints, allowing the identification of specific molecules without labeling.

Owing to signal weakness, however, data collection for Raman spectroscopy in its basic form is slow and therefore impractical for most microscopy applications. A modification of this technique, coherent anti-Stokes Raman scattering (CARS), has been used to address this issue by illuminating the sample with a 'pump' laser beam and a Raman-shifted 'Stokes beam' simultaneously. The beat frequency between the two laser lines resonates collectively with all molecules that feature the targeted vibrational energy level in the common laser focus. The microscope detects the resulting (anti-Stokes) blue-shifted light, which can be easily separated from the laser lines or red-shifted background fluorescence. As the anti-Stokes intensity is quadratic to the number of target molecules, signal levels are increased dramatically over that of spontaneous Raman scattering.

SRS uses the same laser beams as CARS but looks directly at the Raman scattering stimulated and amplified by the pump-Stokes beam combination. In SRS, the stimulated Raman light cannot be separated from the laser light by its wavelength because it is identical to that of the Stokes laser beam. However, the transfer of energy from the illuminating light into vibrational energy of the sample molecules manifests itself in a subtle loss of power in the probe beam (stimulated Raman loss) and a similar gain of power in the Stokes beam (stimulated Raman gain). To separate the signal from random fluctuations in the laser power, the Stokes laser beam is periodically switched on and off on a sub-microsecond time scale. The Raman signal, which varies at the same modulation frequency, can now be filtered out electronically and the enhanced detection sensitivity of only a few thousand molecules per sub-micrometer focal volume enables biological studies[3].

The advances enabled by SRS microscopy make it a powerful and useful tool, with features similar to those of fluorescence microscopy, which other Raman spectroscopy variants, such as CARS lack in this combination. First, the recorded signal is proportional to the concentration of the targeted molecules, and the background signal is very low, which is crucial for quantification. Second, the SRS effect is constrained to the common focus of the two laser beams, enabling three-dimensional laser-scanning microscopy. Moreover, by using reference Raman spectra, the molecules for visualization and quantification can be easily selected by choosing the corresponding pump wavelength.

The ability to select target molecules for imaging is particularly useful for the analysis of neutral lipids, which contain large amounts of aliphatic fatty acid side chains with many chemical bonds adding to the Raman shift (such as CH_2). The fatty acids in neutral lipids are mainly esterified to glycerol (triacylglycerols) or to sterols (sterol esters). In cells, these lipids are found primarily in lipid droplets, which are organelles that are bounded by a phospholipid monolayer and decorated with specific proteins[4,5]. Despite their nearly universal presence in cells, relatively little is known about lipid droplets. Even less is known about why different cell types, such as adipocytes, liver or muscle cells, have dramatically diverse capacities to store different neutral lipids. Moreover, neutral lipid stores in multicellular organisms are subject to complex and coordinated physiology. How this is achieved also remains largely unknown.

To unravel the regulation of fat metabolism in complex organisms, several studies have used conventional light microscopy with genetic screens in yeast, worms and fruit flies to identify genes that govern fat storage[6–11]. Many of these screens used relatively unspecific lipophilic dyes (such as 4,4′-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), Nile Red or Oil Red O), which partition into the hydrophobic phase of the lipid droplet core, resulting in a signal that is detected by fluorescence light microscopy. Despite the usefulness of these dyes for visualizing and studying lipid droplets, they also stain other cellular structures, particularly under some conditions used to visualize fat in whole animals. In one report, for example, the majority of the Nile Red signal was found in lysosomes rather than in lipid droplets[12].

Wang *et al.* applied the power of SRS microscopy to detect lipids in this type of genetic screen[2]. These investigators measured neutral lipids in a pilot RNA interference–based genetic screen of *Caenorhabditis elegans*, and the SRS method allowed them to identify lipid droplets with great sensitivity and specificity. In a screen of selected cell-surface and nuclear hormone receptors, they identified eight new genetic regulators of fat storage, including the worm homolog of natriuretic peptide receptor. Notably, each gene has a human ortholog that is broadly expressed in tissues, including neurons.

This study[2] illustrates beautifully how an advance in methodology can move a field forward, and it promises much more. In fact, by combining SRS with other systems-type approaches—such as genetic screens, genome-wide association studies and better analytic methods to measure lipids globally (such as mass spectrometry)—the stage is now set to unravel the most important players in fat metabolism and its regulation. Moreover, SRS is a flexible methodology that can be applied to study different molecules. It is likely therefore to rapidly gain importance and more widespread use. Given its great precision, investigators will likely embrace SRS microscopy, allowing them the confidence to know that 'what you see is what you get'.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

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