The intrinsic Raman response of chemical species or biological components within a complex heterogeneous system, e.g. a living cell, offers an unparalleled opportunity to not only noninvasively map their chemical composition in space but also to follow their chemical and physical dynamics in real time by coherent anti-Stokes Raman scattering (CARS) microscopy. In particular, the application of high-sensitivity multiplex CARS microspectroscopy, which offers the possibility for space- and frequency-resolved vibrational spectroscopy with high detection sensitivity, high spatial resolution, and three-dimensional sectioning capability, provides access to the full wealth of the spectroscopic information content of macromolecular objects on the sub-micron length scale with spectra acquisition times per pixel typically below 100 milliseconds. Combined with a quantitative analysis of CARS spectra per image pixel, this vibrational microspectroscopy potentially provides a powerful functional imaging modality available to study biological function occurring on time scales of seconds to hours.

To demonstrate this potential of CARS microscopy, we studied the slow time evolution of lipid metabolism in individual intracellular lipid droplet organelles inside a living NIH3T3-L1 adipocyte cell. We activated lipolysis in lipid droplet organelles by chemical stimulation of the cell, and monitored the dynamic changes in their C-H stretching Raman response in the high-wavenumber region during incubation for at least 60 minutes. This in vivo study demonstrates the feasibility of functional CARS microspectroscopy for the examination of the chemical dynamics involved in biological processes under physiological conditions, which are currently not possible to address with conventional techniques in a non-destructive manner.