

## **Dual-CARS microscopy**

**Madeleine Åkeson, Christian Brackmann, and Annika Enejder**

**Molecular Imaging and Biotechnology  
Department of Chemical and Biological Engineering  
Chalmers University of Technology  
Fysikgränd 3, S-412 96 Gothenburg, Sweden  
enejder@chalmers.se**

We present the concept of dual-Coherent Anti-Stokes Raman Scattering (CARS) microscopy for label-free imaging of molecules in living cells. The use of three synchronized laser pulses in a dual-pump/dual-detection configuration enables imaging of two species with different molecular vibrations simultaneously, as well as the acquisition of images free of non-resonant background. We show the power of the method by imaging deuterated nonadecane slowly diffusing into a suspension of living yeast cells in medium, clearly distinguishing the medium and cells from the D-nonadecane by probing the CH<sub>2</sub> and CD vibrations. In addition, images of lipid stores in living *C. elegans* nematodes free of non-resonant background are shown. This results in a significant enhancement of the image contrast, allowing the visualization of emerging, low-density lipid stores in a dauer larva, difficult to distinguish in conventional CARS microscopy. The separation of the non-resonant background is shown to be beneficial also when monitoring molecules with weak vibrational modes. The improved sensitivity obtained is illustrated by probing the C=C vibration in polyunsaturated lipids. This enables the monitoring of the degree of unsaturation of lipids, a central parameter within nutrient research.