nature masterclasses

Online Course in Scientific Writing and Publishing

Delivered by Nature Research journal editors, researchers gain an unparalleled insight into how to publish.

Try a free sample of the course at masterclasses.nature.com

Bite-size design for busy researchers
Subscribe as a lab or institution

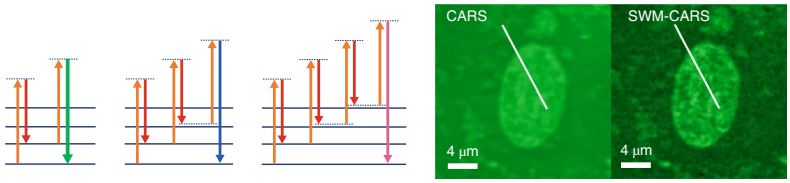
W masterclasses.nature.com
Follow us on LinkedIn
Skills and Careers Forum for Researchers

A80768

IMAGING

Super-resolution Raman imaging

Higher-order processes can be harnessed for super-resolution CARS vibrational microscopy.



Energy-level diagrams of the light–molecule interactions during CARS and HO-CARS imaging, as well as images of buccal cells acquired with CARS (left) or HO-CARS (right), here in the form of six-wave mixing (SWM) CARS. Adapted from Gong, L. et al. *Nat. Photonics* https://doi.org/10.1038/s41566-019-0535-y (2019).

Coherent anti-Stokes Raman scattering (CARS) microscopy is a form of vibrational microscopy that requires multiple photons to elicit the vibration of specific chemical bonds. More specifically, the frequency difference between a pump beam and a Stokes beam leads to molecular vibrations when the frequency difference coincides with the Raman resonance of the targeted molecules. Molecular classes such proteins, lipids and nucleic acids can be distinguished by differences in their Raman resonance signatures.

In analogy to multiphoton microscopy, higher-order nonlinear processes can be detected with CARS microscopy as well, with the benefit of increased resolution. Gong et al. demonstrate such higher-order CARS (HO-CARS) imaging theoretically and experimentally. While CARS elicits Raman resonance once, the researchers postulate that in HO-CARS, Raman resonance is evoked multiple times. As in multiphoton microscopy, excitation volumes in HO-CARS microscopy are smaller and the signal-to-background ratio is increased, compared to standard CARS microscopy.

The researchers experimentally validate these predictions by imaging crystals of the Raman tag 1,4-diphenylbuta-1,3-diyne. They determined experimental resolutions of 230 and 196 nm for second- and third-order CARS, respectively, while the resolution for standard CARS was 328 nm.

Importantly, Raman resonance can be overpowered by fluorescence emission in the sample. However, Raman resonance is narrow-band, and employing adequate narrow-bandwidth filters makes it possible to isolate the typically weaker Raman resonance.

The researchers illustrate the biocompatibility of their HO-CARS technique by imaging HeLa or buccal cells in a label-free manner. They chose resonance frequencies indicative of lipids and/or proteins. As predicted by their initial experiments of non-living matter, the images acquired with HO-CARS exhibited superior resolution and signal-to-background ratios compared to those acquired with standard CARS.

The researchers further note that they did not observe any photodamage during live imaging with HO-CARS and that the excitation powers they used are lower than for alternative Raman-based super-resolution imaging approaches, which had mostly been limited to inorganic materials.

HO-CARS microscopy should be readily implementable by researchers with access to a CARS-compatible microscope, as the technique simply requires tightly focusing the laser beams with a high-NA objective. It will be exciting to apply the HO-CARS approach to a variety of different biological questions and to see the resulting super-resolution images of molecular species in cells and perhaps tissues.

Nina Vogt

Published online: 28 November 2019
https://doi.org/10.1038/s41592-019-0677-3

Research paper
Gong, L. et al. Higher-order coherent anti-Stokes Raman scattering microscopy realizes label-free super-resolution vibrational imaging. *Nat. Photon.* https://doi.org/10.1038/s41566-019-0535-y (2019).