Published in final edited form as:

Nat Chem Biol. 2020 October ; 16(10): 1039–1040. doi:10.1038/s41589-020-00650-0.

Toward spatially resolved metabolomics

Bennett W. Fox, Frank C. Schroeder[✉]

Boyce Thompson Institute and Cornell University, Ithaca, NY, USA.

Abstract

Coherent Raman imaging enables mapping of chemical features at subcellular resolution, setting the stage for tracking lipids and other metabolites in intact living systems.

Despite tremendous progress in metabolomics, lack of spatial resolution severely limits most current approaches to characterizing the chemical makeup of living systems. In a typical metabolomics experiment, homogenized tissue (or entire worms, flies, leaves, etc.) is extracted and then analyzed by LC-MS or NMR, and any information about the spatial distribution, though critically important for understanding the biological roles of any of the detected molecules, is lost. In the case of proteins, the importance of localization is obvious; for example, nuclear and cytosolic localization of a transcription factor may each result in opposite phenotypes. Similarly, different cellular and subcellular localization patterns of metabolites may be associated with considerably different biology. For example, spatial and temporal partitioning of NAD⁺ underlies many of its metabolic and signaling functions¹; tracking the localization and transport patterns of lactate has provided important insights in cancer metabolism²; and subcellular concentration or confinement of secondary metabolites is often essential for their biosynthesis and function³.

In this issue⁴, Chen et al. demonstrate the use of broadband coherent anti-Stokes Raman scattering (BCARS) to image the distribution of different types of lipids throughout the body of the simple model system *Caenorhabditis elegans*. Fat metabolism in *C. elegans* has been studied extensively; many of the involved neural, endocrine, and metabolic pathways are conserved between worms and higher organisms, including mammals. However, most of this work is based on whole-organism lipid analysis or the use of fixative stains such as Oil Red O to measure distribution of lipid-like species across tissues⁵. The latter techniques are primarily based on the lipophilicity of the dyes and thus provide little information about the chemical nature of the stained lipids.

Since the advent of high-resolution NMR spectroscopy, vibrational spectroscopy has lost much of its importance for routine compound identification, though most college analytical chemistry classes retain an infrared (IR) spectroscopy module teaching the characteristic IR frequency ranges indicative of different types of bonds. However, as part of a multifaceted renaissance of vibrational spectroscopy, the development of narrow-spectrum coherent

[✉] schroeder@cornell.edu.

Competing interests

The authors declare no competing interests.

Raman imaging (CRI) suggested that it may become possible to employ vibrational spectroscopy to resolve chemical identity at high spatial resolution in living systems. Chen et al. now show that BCARS provides the sensitivity and spatial resolution to discriminate between different closely related chemical species at the subcellular level. Application to intact, anesthetized worms enabled imaging of a portion of the body at half-micrometer spatial resolution. For each pixel, a full Raman spectrum was obtained, covering most of the classical IR range from 600–3,100 cm^{-1} , which then allowed the characterization of macromolecular lipid droplets. By way of comparison, half-micrometer spatial resolution would correspond to approximately 50 individual spectra (in one dimension) across the diameter of a single HeLa cell, which could conceivably resolve the nucleus, the Golgi, and other cellular features.

Lacking specialized adipocytes, *C. elegans* stores the majority of fats in non-membrane-bound lipid droplets, which are variably sized protein-containing particles localized primarily in intestinal and epidermal cells. CARS imaging now revealed that these lipid droplets are not uniform and instead fall into chemically distinct classes, including large droplets that consist predominantly of lipids, and smaller droplets that, in addition to lipid-derived Raman resonances, also showed strong signature signals of proteins, including amide I stretching and phenylalanine ring breathing (Fig. 1). Taking advantage of the ability to differentiate between the Raman resonances of C–H and C–D bonds, the authors then applied BCARS to study dynamic metabolic flux. By feeding deuterated oleic acid in a pulse-chase experiment, they investigated fatty acid turnover in the different types of lipid droplets, which showed that turnover is fastest in the smaller, protein-rich particles abundant in the intestine. Additional experiments with an egg yolk transporter mutant suggested that these intestinal lipoprotein particles function as lipid delivery vehicles to the developing progeny. In contrast, the slow turnover rates observed for the larger fat particles, which predominate in the hypodermis, indicated that such large droplets serve as lipid storage depots.

Impressively, BCARS allowed characterization of the chemical make-up of the different types of droplets in considerable detail, revealing strong resonances indicative of triglycerides in the large droplets, whereas spectra of the lipoprotein particles lack the ester resonance and instead show signals suggestive of both saturated and unsaturated free fatty acids. Although the characterization of chemical structures using BCARS is still somewhat cursory, further improvements to BCARS sensitivity and resolution may enable the identification and tracking of distinct types of metabolites. Furthermore, calibration of different resonances may permit translation of spectral intensities into truly quantitative maps for different chemical species.

BCARS thus joins a growing set of tools to tackle the challenge of characterizing spatial and temporal patterning of metabolites, complementing other techniques such as desorption electrospray ionization-imaging mass spectrometry (DESI-IMS)⁶. Of particular appeal is the notion that BCARS can be used to study living, intact specimens over extended periods of time, providing direct insight in the dynamics of metabolic processes.

References

1. Cambronne XA & Kraus WL Trends Biochem. Sci 10.1016/j.tibs.2020.05.010 (2020).
2. Niu X, Chen YJ, Crawford PA & Patti GJ Cancer Metab. 6, 19 (2018). [PubMed: 30559963]
3. Kistler HC & Broz K Front. Microbiol 6, 68 (2015). [PubMed: 25709603]
4. Chen W-W et al. Nat. Chem. Biol 10.1038/s41589-020-0565-2 (2020).
5. Escorcía W, Ruter DL, Nhan J & Curran SP J. Vis. Exp 2018, e57352 (2018).
6. Parrot D, Papazian S, Foil D & Tasdemir D Planta Med. 84, 584–593 (2018). [PubMed: 29388184]

Fig. 1 |. Imaging of anaesthetized worms via BCaRS reveals chemically distinct classes of lipid droplets in the intestine and epidermis and enables the tracking of lipid turnover. Structural features detected by BCaRS are highlighted red.