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Label-free molecular imaging of an arbuscular mycorrhizal fungus using multiplex CARS microspectroscopy

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Arbuscular mycorrhizal fungi (AMF) have symbiotic interactions with the roots of many land plants, in which they provide plants with phosphorus from the soil and, in turn, receive the products of photosynthesis from plants [1]. Toward elucidation of the mechanism of this symbiosis, it is crucial to visualize the localization and dynamics of various substances involved in a mycorrhiza, but label-free imaging of AMF has remained largely unexplored. In this study, we performed fast, label-free molecular imaging of the spores and hyphae of the AMF *Rhizophagus irregularis* using broadband multiplex coherent anti-Stokes Raman scattering (CARS) microspectroscopy.

R. irregularis was grown on 1.5% agar medium for 9 days. Mapping measurements were performed on 25 × 25 μm^2 regions using a laboratory-built multiplex CARS microscope [2] with the pump (ω_1) light at 1064 nm and the supercontinuum Stokes (ω_2) light ranging from 1080–1600 nm [3]. The power of the ω_1 and ω_2 light was 10 and 1 mW, respectively. The obtained CARS spectrum was converted to Im[$\chi^{(3)}$] spectrum equivalent to the spontaneous Raman spectrum using the maximum entropy method. The exposure time per point was 0.2 s and the step was 0.5 μm.

Figure 1a, b displays the CARS imaging results of *R. irregularis* spore and hypha, respectively. By using the Raman bands characteristic of unsaturated lipids and polysaccharides, we successfully visualized the cell walls of the spores and hyphae, lipid droplets accumulated in the spores, and the interior of the hyphae without staining. This method is applicable to molecular imaging of mycorrhizae in plant roots, which is currently underway in our laboratory.

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[2] R. Sasaki et al., *J. Phys. Chem. B*, **127**(12), 2708-2718 (2023)
[3] H. Segawa et al., *Opt. Express*, 20(9),9551-9557(2012)

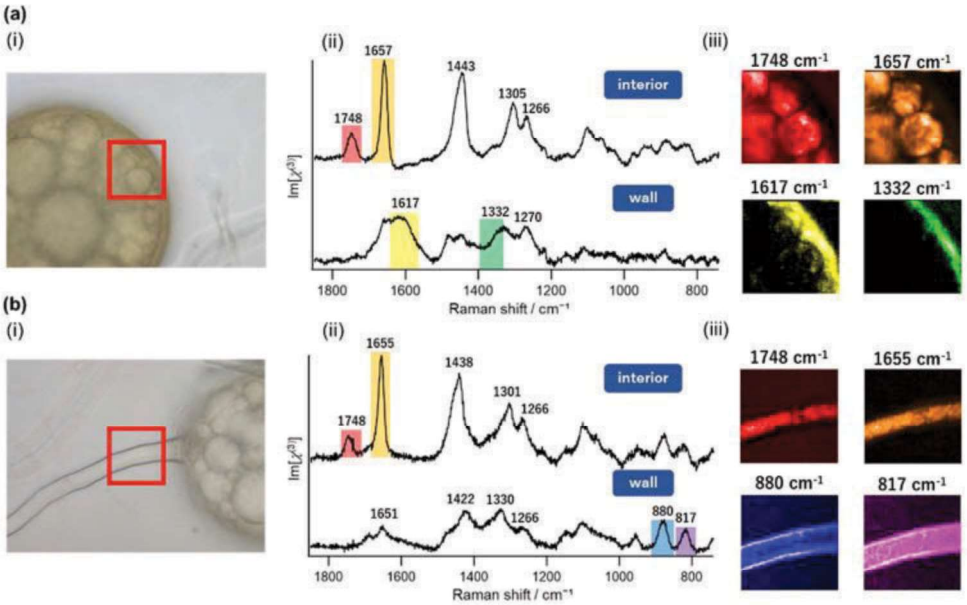

Figure 1 Multiplex CARS imaging of a spore (a) and a hypha (b) of the arbuscular mycorrhizal fungus *R. irregularis*: (i) Optical micrographs showing the imaged regions with red squares; (ii) representative Im[$\chi^{(3)}$] spectra measured at specific locations in the spore and hypha; and (iii) CARS images at different Raman shifts.