Lable-free images in ex-vivo cultured retina by coherent anti-stokes raman scattering (CARS) microscopy

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Nonlinear spectroscopic imaging modalities have the potential to visualize cellular organelles and tissue architecture with molecular specificity. Coherent anti-Stokes Raman scattering (CARS) microcopy has recently emerged as the most viable means for 3D chemical imaging of tissues, which works by probing intrinsic molecular vibrations, which obviates the need to label target molecules and fix specimens. [1] Herein, we investigated the cellular and non-cellular structures of ex-vivo cultured mouse sensory retina without fluorescent labeling by CARS microscopy. The ex-vivo cultured sensory retinas were imaged through the overlying vitreous by CARS microscopy. Images from ganglion cell layer to photoreceptor outer segment were sequentially analyzed by depth-wise application of CARS microscopy. Cellular structures in ganglion cell layer, inner nuclear layer, and outer nuclear layer and non-cellular structures in inner plexiform layer, outer plexiform layer, and photoreceptor outer segment were clearly identified, where highly selective CARS images were detected in sub-cellular organelles with lipid-rich plasma membrane. Interestingly, distinct vibrational peaks in CARS spectra from 2700 to 3100 cm^{-1} were observed, where is speculated that carbon–hydrogen bonds would be abundant in lipid compared to the surrounding tissues. Our result suggest that CARS imaging could provide precise morphologies of retina with correlative chemical information without exogenous labeling. With technical advancements, CARS microscopy would be applied to lipid-associated retinal disorders and the preclinical drug screening.

KEY WORDS: Coherent anti-stokes raman scattering, ex-vivo culture, label-free, retina

[1] S.H. Kim; J.Y. Lee; E.S. Lee; B.S. Lee; J.E. Park, and D.W. Moon. “Multiplex coherent anti-stokes Raman spectroscopy images intact atheromatous lesions and concomitantly identifies distinct chemical profiles of atherosclerotic lipids”, *Circ. Res.*, **106**, 1332-1341 (2010).