Raman probe coupling proximity labeling technique for organelle-specific bioimaging

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Raman microscopy has a high potential to probe intracellular environments thanks to its spectral sensitivity to local electric fields. However, the extremely weak signal of Raman scattering (~10^{15} times weaker than fluorescence emission) have hindered its application to molecular profiling of intracellular organelles. To address this challenge, we comobined a proximity labeling approach with spontaneous Raman microscopy to enable bioimaging of various organelles within living cells. Genetic incorporation of APEX2 allows for site-specific labeling of the Raman probe. The precise localization of new diynes probes within the organelles of interest by proximity labeling, the strong signal at the cell silent frequency window (1800-2800 cm^{-1}), and photostability of the probes enable visualization of various intracellular compartments at high-speed imaging, surpassing the long acquisition time/accumulation of conventional confocal Raman microscopy. An organelle-specific Raman image was constructed within a few minutes (<5min) using the peak intensity at 2213 cm^{-1}, which was assigned to the diyne stretching mode under irradiation with visible light at 532 nm with low laser power of (<10mW) and an integration time of 1s. A clear colocalization of both diyne stretching mode and the pyrrole breathing mode of cytochrome C, a specific marker of mitochondria, confirmed the high specificity of Raman mapping (**Figure 1**). The Raman signal of the diyne stretching mode was an order of magnitude stronger than the signal of the pyrrole breathing mode of cytochrome C and alkyne mode of EdU, a commonly used vibrational probe for nuclear imaging. Our proximity labeling Raman microscopy enabled organelle-specific Raman mapping with much shorter acquisition time and weaker laser power than those for cytochrome C and EdU, which is much less toxic to live cells.

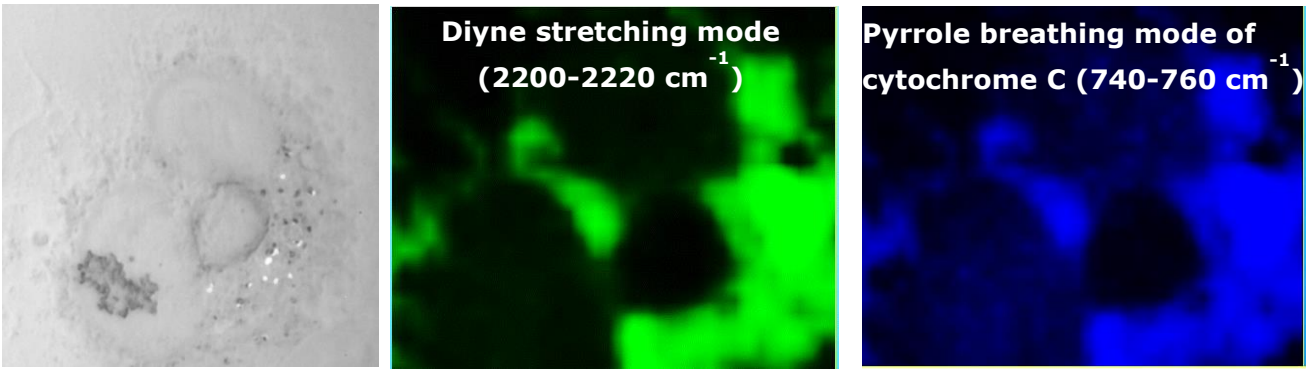

Figure 1. Bright field image and Raman images show colocalization of both diyne stretching mode and the pyrrole breathing mode of cytochrome C