

LIVE-CELL IMAGING AND SPECTROSCOPY OF REACTIVE OXYGEN SPECIES IN ACTION

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Cells are under oxidative stress as the level of reactive oxidative species (ROS) is elevated. Owing to its relatively high reactivity, ROS can cause structural and functional derangements of vital biomolecules resulting in cellular oxidative injury. The assessment of cellular injury induced by ROS has been a challenge as conventional methods are limited either by low sensitivity or lacking molecular and temporal specificity. Advance in laser technology has stimulated the investigation of biomedical processes at the level of a single cell. We recently employed laser tweezers assisted single-cell Raman spectroscopy to monitor the biochemical transformation of a single biological cell under oxidative stress [1]. By means of sequential spectral measurements, we identified lipid peroxidation occurring during oxidative injury and demonstrated the therapeutic effect of ascorbic acid to such injury. Very recently, we further extended the approach to investigate intracellular processes [2]. By using autofluorescence imaging and Raman microspectroscopy, we demonstrated label-free visualization of NADPH oxidases in living macrophages that are undergoing phagocytosis and quantitative assessment of phagocytic-ROS-caused oxidation occurring in intraphagosomal yeasts with no stains. Our work suggests the combined use of Raman microspectroscopy and autofluorescence imaging possesses great potential in the characterization of cellular processes *in vivo*.

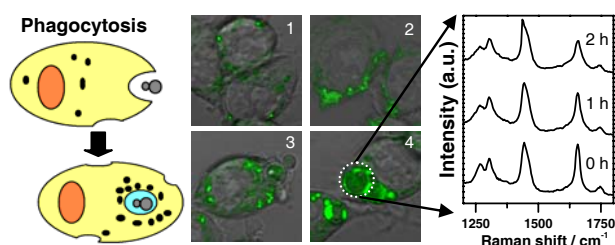


Figure 1: Autofluorescence imaging and sequential Raman spectra of single ingested yeast in a living macrophage.

[1] W. T. Chang et al, “Real-time molecular assessment on lipid peroxidation of single cells using Raman spectroscopy” *J. Raman Spectroscopy* **40**, 1194-1199 (2009).

[2] W. T. Chang et al, “Spatiotemporal characterization of phagocytic NADPH oxidase and oxidative destruction of intraphagosomal organisms *in vivo* using autofluorescence imaging and Raman microspectroscopy”, *J. Am. Chem. Soc.* **132**, 1744-1745 (2010).