Raman microspectroscopy: From single cell imaging to diagnostics

Hemanth Noothalapati
Faculty of Life and Environmental Sciences, Shimane University, Japan
nvhnag@life.shimane-u.ac.jp
Hemanth.n@che.iith.ac.in

Biological processes naturally have intricate designs making it difficult to study in detail. In fact, many areas in biology including intracellular dynamics and its biochemistry still largely remain unexplored due primarily to the lack of appropriate tools. Here, I discuss a few examples of how Raman spectroscopy and microscopy aided with multivariate statistics, AI/ML can gain fundamental and otherwise unobtainable biological information to decode some of nature's complex designs.

Single cell Raman imaging: We present for the first-time *in vivo* time lapse Raman imaging, coupled with stable-isotope (^{13}C) labelling, to reveal dynamic proteome localization to lipid droplets in single living *Schizosaccharomyces pombe (S. pombe)* cells. We further developed a label-free method based on confocal Raman microscopy to visualize distributions of various polysaccharide components of fungal cell and spore wall. We successfully probed fibril formation and therapeutic targeting of amyloid-like structures in a yeast model of adenine accumulation.

Probing cellular biochemistry *in vivo*: We show that combination of stable isotope probing (SIP), Raman micro-spectroscopy and multivariate curve resolution (MCR) analysis can serve as a valuable approach in metabolomics research by studying ergosterol biosynthesis in single living fission yeast cells. We further visualized wax ester fermentation in single Euglena cells using Raman microscopy and MCR analysis and obtained chain length-specific information and intracellular distribution images of the produced esters. Accumulated esters in Euglena were particularly identified to be myristyl myristate (C28), a wax ester candidate suitable to prepare drop-in jet fuel. Recently, we are interested in pharmaceutical applications, i.e., imaging drug and its effect at single cell level. I will discuss some results on antifungal agents.

Raman diagnostics: The prevalence of eosinophilic esophagitis is increasing. We used Raman spectroscopy as a non-invasive tool to study esophagus of mouse models in which esophagitis is induced artificially by injecting IL-33. We distinguished eosinophil from other types of white blood cells and then successfully developed a Raman spectroscopy based diagnostic technique to detect the existence of eosinophils in esophageal mucous membrane. Recently, we identified Raman spectral markers and developed a method to objectively discriminate breast cancer and normal mammary epithelial cells in a label-free manner which will be introduced briefly in the meeting. Further, I will discuss how we can build reliable data discrimination models based on explainable AI.