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Multimodal label-free imaging and complementary imaging pathways based on different scattering modes

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1. Introduction

We develop imaging modes for Raman measurement of living cellular changes in response to immunological stimulus. Using a combination of simultaneous Raman scattering and quantitative phase imaging, two complementary but incomplete views of the sample are attained. It is then challenging to determine how these two sets of available data should best be combined, or analyzed, in terms of providing the most useful information to the imaging scientist.

2. Multimodal Raman

Raman spectroscopic imaging allows label-free observation of the molecular distributions in the cell. Given sufficient signal-to-noise ratio in the measurements, the spatially resolved distributions of different molecular compounds in a living cell may be readily observed [1]. In practice, the limitations on the ability to discriminate dynamic changes in live cells rests on the low visibility of the spectral signals and these are in turn usually limited by the total amount of laser power which can be applied to the sample. Therefore, against these fundamental physics-based limitations, a number of approaches have been implemented. Coherent excitation approaches or nanoparticle enhancement have shown the possibility of drastically increasing the detected signal strengths, but usually at the cost of changing some of the nature of the detected information. Both coherent based Raman and surface enhanced Raman techniques provide information that is spectrally different or more limited when compared to spontaneous Raman methods.

Simultaneous label-free multimodal imaging

We built a multimodal setup, with the expectation that combining quantitative phase imaging by coherent illumination with Raman by spectrally separating the two modalities. This provides the very significant advantage of having a rapid view of the sample in phase during the time that the Raman signal is acquired. The resulting data allows us to track morphology and structural changes (or motion) in the cell by phase while tracking molecular signatures in the slower Raman modality. In normal Raman imaging, while temporally and spatially resolved Raman images can be acquired, the overwhelming variety of proteins, lipids and other molecules makes quantitative determination of the source of the Raman signal difficult. The additional modality allows us to use the phase information to help unravel the complex Raman signal [2].

The Raman signals are composed of the spectral signature of each molecule in the focal volume depending on its concentration and scattering cross-section. The total signal also includes background noise from the detector, and inherent noise due to the low number of photons. Detection or determination of the changes in one particular molecule is usually very difficult however, some cellular molecules have absorbance at the incoming light wavelength and produce resonant Raman signals, appearing above the background.

The phase signals represent the total amount of retardation of the light passing through the sample and the contrast in the phase imaging mode then depends on the optical density of the sample. Surprisingly, the phase and Raman imaging modes, even though based on different physical interactions, share enough similarity to recognize similar features in both. However, on closer analysis, we found that the molecular contributions to each imaging mode are different, with phase contrast depending more on protein distributions and the Raman mode contrast being more sensitive to lipid distributions, although protein, lipid, nucleic acid, and other contributions are present in both modes.

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