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Multimodal Raman Imaging and Analysis for Label-free measurement of cellular structure and dynamics

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1. Introduction

We develop protocols for Raman microscopy of living cellular changes in response to immunological stimulus and also attempt to quantify molecular structural information in the cell by combining the information available through complementary label-free imaging modalities.

2. Multimodal Raman

Raman spectroscopic imaging allows label-free observation of the molecular distributions in the cell [1]. While challenging due to weak light emission, and having an inherent scattering cross-section orders of magnitude weaker than fluorescence-based emission, the Raman spectra carries information characterizing the ensemble of molecular bonds in the sample.

Raman imaging

It is therefore possible to use Raman imaging to measure cell structure and to track molecular changes in the cell, and since the imaging requires no label or assumptions about the sample it allows a more holistic view of the cell and has enormous potential to unlock hidden dynamics in a during biological reactions

. A major challenge is to interpret the measured signals, and an additional limitation is the typically weak signals obtained. An approach to alleviate this limit is to add additional information by using a multimodal approach. Adding particles, fluorescent dyes, or even ordinary light sources for widefield imaging typically swamp the weak Raman signal and are therefore generally unsuitable.

We therefore chose to implement a multimodal setup, 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.

The 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

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Analysis of target molecules and diagnostic approaches

In malarial infection, the malaria parasite cleaves the hemoglobin in red blood cells, leaving free heme which is biocrystallized into hemozoin, a heme-based nanocrystal. Heme, is strongly resonant at 532nm, and the Raman analysis can be used to detect slight changes in the presence of Heme, leading to the use of Raman

The malaria parasite synthesis of hemozoin changes the heme spectral response only slightly, and hemozoin is also relatively easy to detect compared to other components in the cell. When the blood cells break down, the hemozoin can reach other cell types and is known to trigger an immune reaction in macrophage cells [2], leading to significant interest in the related cellular changes.

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