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Enhancement of the lateral resolution of Raman microscopy by use of structured illumination

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1. Introduction

Recent developments in optical microscopy especially fluorescence microscopy have pushed the limits of spatial resolution below the diffraction limit, to as low as several nanometers. Most of these super-resolution techniques, however, cannot be adapted to Raman microscopy because their working principle is based on manipulating the fluorescence emission properties of the sample. On the other hand, structured illumination microscopy (SIM) utilizes the Moire effect (interference of the structured light pattern with the sample structure) to double the spatial resolution [1] and therefore does not impose particular optical properties on the sample, making it a feasible technique for Raman microscopy.

Here, we show the enhancement of the lateral resolution of Raman microscopy by use of structured illumination [2]. The structured light pattern was introduced into a conventional slit-scanning Raman microscope along the longitudinal direction of the line illumination, where the resolution is expected to be enhanced after SIM image reconstruction.

2. Optical setup and image reconstruction

The optical setup for structured line illumination (SLI) Raman microscopy can be described as follows. The 532-nm excitation laser passed through a phase grating forming two beams that recombined at the sample plane to form the interference fringes in the line-shaped focus. The Raman scattering from multiple points in the line were then recorded simultaneously by a spectrometer equipped with a cooled CCD camera, and the line illumination was scanned across the sample to form a Raman image. To reconstruct the SLI image with enhanced spatial resolution, three Raman images were acquired at three different fringe phases and processed using a SIM image reconstruction method along the slit direction.

3. Results

Using 500-nm diameter polystyrene beads as sample, we demonstrated the increase in the spatial resolution of SLI Raman microscopy. Figure 1 shows the Raman images of the bead sample obtained by the SLI Raman microscope and conventional line-illumination (LI) Raman microscope. The images were reconstructed using the Raman shift of 1003 cm^{-1}. The individual beads are clearly more resolved in the SLI Raman images than in the LI Raman images.

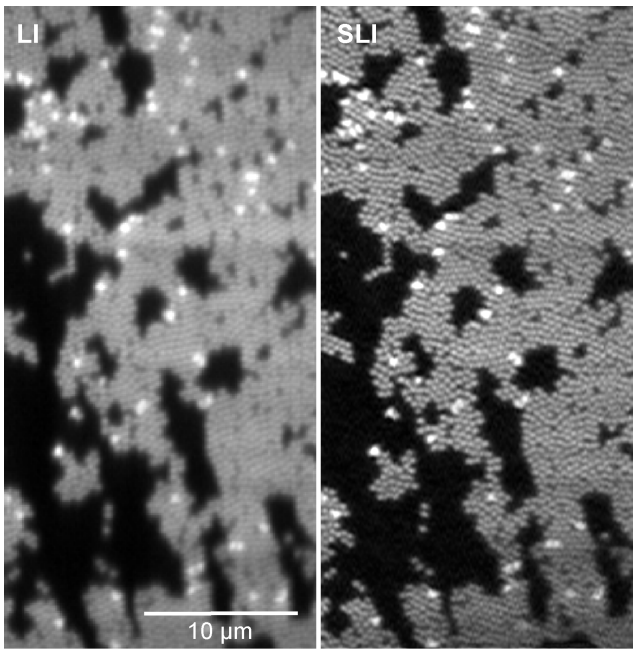

Figure 1. Raman images of polystyrene beads obtained by LI and SLI Raman microscopy.

SLI Raman imaging of carbon materials and mouse brain tissue also showed finer spatial details, demonstrating the applicability of SLI Raman microscopy to more complex samples. Due to the resolution enhancement, the distinct Raman spectra obtained from adjacent pixels of the sample reveal the improved spectral discrimination capability of SLI Raman microscopy.

In this work, we presented super-resolution spontaneous Raman imaging by use of structured illumination. In many practical applications, the spatial resolution of SLI Raman microscopy can easily reach the theoretical limit of confocal Raman microscopy, which is often limited by low signal-to-noise ratio. Therefore, SLI Raman microscopy is expected to further expand the applications of Raman microscopy in various research fields [3].

References

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