LASER-BASED ACTIVE-ILLUMINATION HYPERSPECTRAL MICROSCOPY WITH MULTI-MODAL IMAGING ANALYTICS

Jingting Li* and Wei-Chuan Shih
NanoBioPhotonics Group
Department of Electrical and Computer Engineering
University of Houston
Houston, TX 77204-4005

Abstract

We present a parallel Raman microspectroscopy scheme for simultaneously collecting Raman spectra from multiple points by projecting a series of multi-point laser illumination pattern using a spatial light modulator (SLM). The scheme enables us to collect Raman spectra within 80×80 μm² field of view from as many as 100 points simultaneously per illumination pattern. With a series of patterns calculated from the bright field image considering the vertical separation distance between two adjacent particles, we can cover all the particles in one sample without overlapping spectra information.

Introduction

Raman spectroscopy is a powerful tool for compositional analysis via inelastic light scattering. Conventional Raman spectroscopy requires a long acquisition time since it is a weak phenomenon. We present a Raman spectroscopy scheme to automatically generate a series of illumination patterns to avoid vertical overlapping just from the bright field image of a translucent sample and acquire Raman spectra with high speed.

Analysis

To acquire a Raman spectra map of a sparse sample is time-consuming and inefficient. Instead, using SLM to active illuminate the sample with a series of patterns can achieve higher efficiency without mechanical moving or waste of laser power by scanning areas without valid sample. To avoid overlapping of spectra, on the direction vertical to spectra, the distance between two adjacent illuminated sample points should not be less than its size. Thus we can split all the points into subgroups within which there is no overlapping.

Methods

Sample used is 2μm polystyrene beads on a coverslip. We use a flat silicon wafer to verify the positions of Illumination patterns. First a visual image is taken by camera1. A program module calculates the centroids of bead and separates them into subgroups. Then we generate the accordingly holograms of each pattern by a module written with LabView. The laser wavefront is modulated by an SLM to generate the desired pattern. Illuminated sample spectra is taken by camera2.

Results

Shown in Fig.1 (a) is a bright field image of 1413 polystyrene beads of 2μm diameter within 80 × 80 μm². The centroids of all beads are separated into 45 patterns that covers
all the beads, of which first 3 are shown in Fig.1 (b). Then SLM displays holograms as shown in Fig1 (c) to modulate laser into desired illumination patterns while computer controlled camera2 takes Raman spectra of each illumination pattern.

Fig. 1. (a) Bright field image of 1413 polystyrene beads; (b) Diagram of the first three illumination patterns; (c) Holograms of first three patterns.

Conclusions

The scheme we present can achieve active Illumination in order to acquire non-overlapping Raman spectra from sparse samples.

References