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Jeongyong Kim, Standardization of Spatial Resolution of Analytical Optical Spectroscopy

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Standardization of Spatial Resolution of Analytical Optical Microscopy

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Spatial resolution is one of the main specifications of confocal microscope. However, the definition and the measurement procedures largely vary depending on the manufacturer of the confocal Raman and confocal fluorescence microscope, therefore the general assessment of the spatial resolution has been limited. We try to provide standardized protocols that describe the measurement of the spatial resolution of analytical confocal microscopes by using small object method.

1. Introduction

Confocal fluorescence microscopes (CFMs) are laser scanning confocal microscopes operated in a fluorescence imaging mode to obtain a fluorescence image of a sample. Fluorescence is the light emitted by a molecule or solid lattice during relaxation after undergoing photon absorption and electronic excitation. The fluorescence wavelength, intensity, and spectral shape are specific to the electronic structure of the material; therefore, fluorescence spectroscopy and imaging techniques are useful for chemical characterization analysis. Among the optical imaging and spectroscopy tools, CFM yields a high spatial resolution that is advantageous for analyzing nanomaterials and thin films. The spatial resolution is one of the most important performance factors for a CFM.

Raman microscope is a very useful chemical characterization tool that spatially visualizes the Raman activity of the sample with the diffraction-limited high spatial resolution. While the spatial resolution is a key specification that strongly represents the performance of manufactured Raman microscopes, it is often characterized in different ways. The spatial resolution of a technique refers to the maximum resolvability of two adjacent objects. This value is often characterized in different ways by the manufacturers. Thus, convenient and effective method for measuring the lateral resolution of a CFM and confocal Raman microscope is demanded.

2. Small Object Imaging Method

A very small object may be imaged to estimate the lateral resolution of a CFM. This approach is advantageous because one can obtain a two-dimensional profile that directly shows the point spread function (PSF) of the CFM from a single CF image. The lateral resolution can be defined as the full width at half-maximum (FWHM) of the PSF. Because the finite size of a small object contributes to the observed size of the object in the CFM image, the observed image of the object represents the convolution of the PSF and the spatial distribution of the small object. A wide range of samples may be used in this method, including fluorescent nanoparticles, such as light-emitting polymer nanoscale beads or

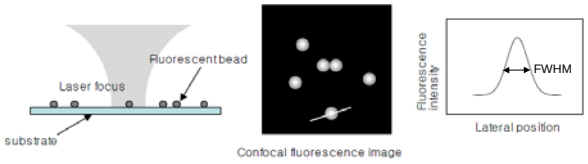

Fig. 1 A fluorescence image may be obtained, and the FWHM of the cross-sectional profile of a single bead is used to estimate the lateral resolution of a CFM instrument.

quantum dots (QDs). Fig.1 shows the concept of the CFM measurement of fluorescent bead and the extraction of a line profile for the FWHM estimation. This idea can be applied equally to the estimation of lateral resolution of confocal Raman microscope.

If the size of the fluorescent nanoparticle is comparable to the size of the PSF in a CFM, a deconvolution process for deducing the actual PSF of the microscope is required. Deconvolution complicates the process of characterizing the PSF. The nanoparticles used here should be as small as possible; however, small particles produce a weaker fluorescence signal that can degrade the signal-to-noise ratio in a CFM image. Assuming that the PSF has a Gaussian profile, a particle less than one-fourth the size of the PSF will contribute less than 1% to the resultant image [1]. Considering the uncertainty of measuring the FWHM of a particle, this discrepancy may be taken as negligible. One-fourth the size of a typical PSF corresponds to 75 nm for a 300 nm PSF, indicating that one can use a 75 nm nanoparticle as a test specimen without worrying about deconvoluting the PSF from the obtained image. The apparent size of the nanoparticle in the image may then be taken as the lateral profile of the PSF. The shape of the fluorescent nanoparticle does not matter as long as the size in any direction of the nanoparticle is less than one fourth of the expected lateral resolution. Note that specified size of the manufactured fluorescent nanoparticles is not always correct with some variation depending on the manufacturer. The confirmation of the size of the nanoparticles by using other high-resolution microscopes, such as atomic force microscope and electron microscopes. The areal density of the nanoparticles is also important, because aggregation or the closed packing of the nanoparticles will hinder the analysis of the single isolated nanoparticle. Several numbers of nanoparticles per 5×5 μm^2 is a recommended density of nanoparticle dispersion.

For Raman microscope, Raman-active nanomaterials should be used, and carbon nanotubes are good candidates. Fig. 2 shows a representative result of measurements of lateral resolution of confocal Raman microscope.

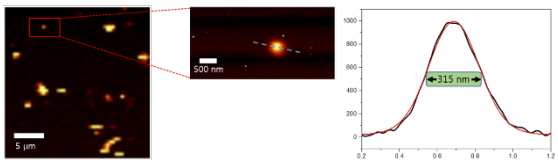

Fig. 2 Extraction of a line profile from a Raman image of dispersed CNTs

For measurement of axial resolution, suspended graphene is an ideal standard specimen, because 1) the thickness of suspended graphene can be made to be substantially smaller than the axial resolution, 2) the dielectric environment above and below suspended graphene is identical. Fig. 3 displays the result of axial resolution using suspended graphene.

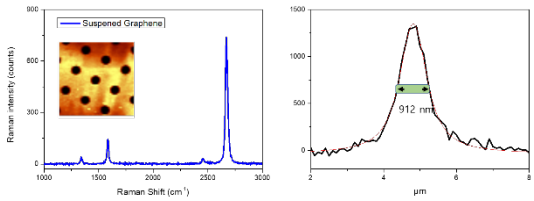

Fig. 3 Axial line profile of 2D Raman band of suspended graphene layer

3. Conclusion

We described the methods of standardized estimation of lateral and axial resolution of analytical optical microscopes. We found that the use of nanosized fluorescent beads, carbon nanotubes and suspended graphene provided simple, convenient yet reliable test specimens for measurement of sub-micron optical spatial resolution.

4. References

[1] Determination of lateral resolution; G. Wilkenig and L. Koenders, Nanoscale calibration standards and methods, Ch. 21 (2005) Wiley-VCH