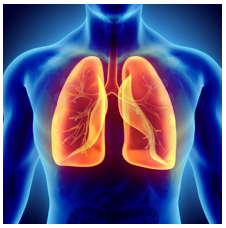
HORIBA Scientific

Raman Spectroscopy

Multimodal Hyperspectral Imaging for Nanotoxicological Applications



Application Note

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Maruda Shanmugasundaram[1], Jamie Uertz[2]

[1]Raman/AFM Applications Scientist, HORIBA Instruments Inc., Piscataway NJ USA, [2]Applications Scientist, CytoViva Inc., Auburn AL USA

Abstract: An imaging platform that combines Raman microscopy with enhanced darkfield and hyperspectral imaging technology was used to study lung tissue sections containing carbon nanotubes. It enabled easy identification of the regions containing the carbon nanotubes followed by spectroscopic characterization of chemical composition.

Keywords: Lung tissue, MWCNT, Raman, EDF, HSI, Nanotoxicity.

Raman-EDF/HSI multimodal imaging

Emerging fields of research such as nanotoxicology, environmental health, and drug design require physico-chemical characterization of nanomaterials in order to examine the health effects of xenobiotic exposure to tissue cultures or model organisms.[1] They could be environmental or occupationally-derived nanomaterials that cause unintended toxicity, or a small molecule or biological drug used for targeted delivery with an intended therapeutic effect. Such substances can be analyzed by Raman microscopy, which is useful for performing physiological, pharmacological, and toxicological assessments. Conventional sampling methods for Raman microscopy use standard brightfield or darkfield imaging in order to identify regions of interest prior to the spectroscopic analysis.

Enhanced darkfield (EDF) imaging by CytoViva enables the detection of nanomaterials in large, complex areas of interest with relative ease compared to standard darkfield imaging. CytoViva's patented EDF illumination maximizes photon density on the sample while minimizing light loss, increasing signal-to-noise by a factor of ten.[2] Hence, it is well-suited to detecting very small exogenous substances present in complex environments such as tissues. Since the EDF technology is integrated with hyperspectral imaging (HSI) by CytoViva and Raman microscopy by HORIBA Scientific, the analyst can explore the substances with EDF to identify the regions of interest, study with HSI to differentiate components based on their optical characteristics (e.g. plasmon resonance, scattering, emission, etc.) and identify chemical components to access chemical composition with Raman spectroscopy. EDF, HSI, and Raman analyses can be performed without having to move or transfer the sample, in a perfectly co-localized tandem fashion.

Here, lung tissue sections from rats exposed to multi-walled carbon nanotubes (MWCNTs) by inhalation[3] were imaged with the combined imaging platform described above. While CNTs are widely used in industry due to their exceptional material properties, chronic exposure in an occupational setting is a key concern. It is well-known that they cause inflammation and pulmonary fibrosis.[4,5] Current research is aimed at studying the pathological mechanism of such diseases in order to develop pharmacological interventions and design safer nanomaterials, for which their easy identification and chemical characterization are needed in complex systems.

Method

Lung tissue sections containing MWCNTs were imaged using the EDF illuminator (CytoViva Inc., Auburn, AL) with the light source coupled via a liquid light guide. The HSI System 1.6 (CytoViva Inc., Auburn, AL) was used to acquire HSI data with a 100× objective (air dry, N.A. = 0.9) and 100 ms exposure per line. Each line comprised of 695 pixels such that a 90 x 90 µm area was scanned with 695 x 695 pixels. Each pixel (128 × 128 nm) contained spatial and spectral information. Spectral information was collected from 400 - 1000 nm with 2 nm resolution. Raman hyperspectral

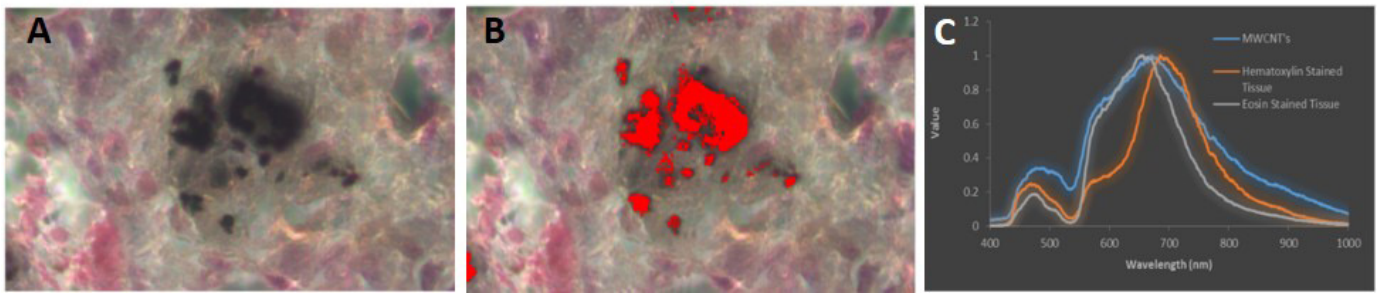

Figure 1: EDF and HSI images of MWCNTs in lung tissue sections. The figure shows a comparison of the EDF and HSI image of MWCNT (A-B, overlaid), and representative HSI spectra of MWCNT, hematoxylin-stained tissue, and eosin-stained tissue (C).

imaging was performed with an XploRA PLUS confocal Raman microscope (HORIBA Scientific, Piscataway, NJ) using 638 nm excitation and the same 100× objective. A total of 20,385 spectra were collected from a ~ 45 µm × 40 µm area with 300 nm step size, followed by polynomial baseline correction and smoothing. The XploRA PLUS is the base system for the combined Raman-EDF/HSI system.

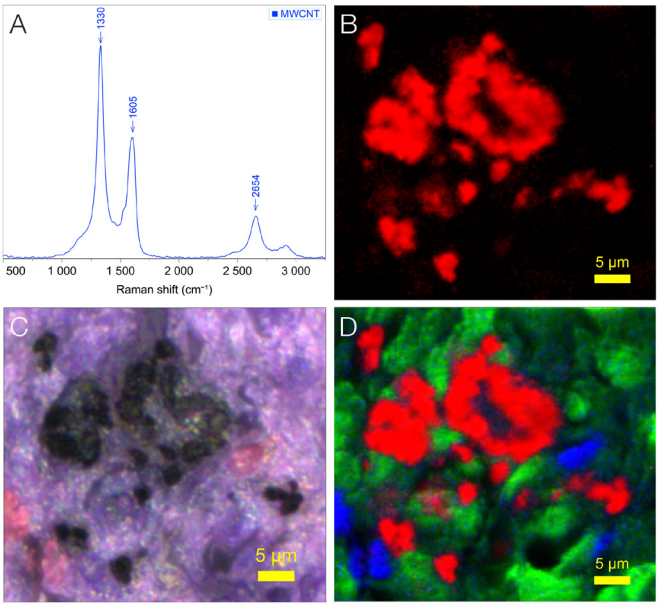

Figure 2: Confocal Raman microscopy. (A) A representative Raman spectrum measured from MWCNTs in lung tissue, (B) Raman image of MWCNTs,(C) corresponding reflected brightfield image showing MWCNTs and stained tissue, and (D) combined Raman image of MWCNT (red), hematoxylin (green) and eosin-stained tissue (blue). Raman images are classical least square (CLS) fitting scores images of representative spectra of individual elements.

Results

Lung tissue of rats exposed to MWCNTs was analyzed by Raman and EDF-HSI imaging. EDF images readily showed the presence of MWCNTs as dark colored areas against the eosin and hematoxylin-stained lung tissue (Figure 1A). This allowed identification of MWCNT for HSI (Figure 1B), and Raman (Figure 2B) imaging. The HSI analysis revealed three components: MWCNTs, eosin-stained tissue, and hematoxylin-stained tissue, all of which had unique optical spectral response to the transmitted light illumination at different wavelengths (Figure 1C).

Raman spectra (Figure 2A) showed peaks assignable to CNTs, such as the disorder band (D-band), around 1330 cm^{-1} which may arise due to out-of-plane C-C vibrations associated with sp^3 structure or another similar symmetry-breaking effect, the graphitic band (G-band) around 1605 cm^{-1} which arises from in-plane C-C vibrations, and the 2nd order D-band (2D-band) around 2654 cm^{-1}.[6] This led to the imaging of the distribution of MWCNTs in the lung tissue (Figure 2B, red). The relative intensity of the D-band is quite high compared to G-band intensity, which has been shown to be the case in MWCNT spectra, as opposed to that of single-walled CNTs, as the presence of multiple layers adds to the defects or impurities.[7]

Spectra collected from the tissue had laser-induced fluorescence due to the presence of the staining agents. Nevertheless, Raman peaks were observed at 1518 and 1613 cm^{-1} which were assigned to eosin- and hematoxylin-stained tissues, respectively. The imaging of these two peaks led to visualizing the distribution of the stained tissue as well (Figure 2D, blue and green). The distribution of the stained tissue can also be seen in the image registration between the optical image and the combined Raman image (Figures 2C-2D).

Summary

A novel, multi-modal imaging platform was developed by combining Raman microscopy with enhanced darkfield – hyperspectral imaging. This method allows for tandem analysis of biological tissues for the presence of nanoparticles based on their optical response, as well as their chemical composition, without having to move or transfer the sample. Presented here is an example which shows imaging of MWCNTs in lung tissue in rich detail. The enhanced darkfield imaging provides easy identification of the foreign material and hence easy sampling for HSI and Raman imaging. Raman analysis allows identification and characterization of the MWCNTs based on their chemical composition. HSI analysis provides orthogonal identification of MWCNTs. This platform is expected to be widely applicable in the study of nanoparticles in biological systems, which is highly relevant to fields of research such as nanotoxicology, environmental health, drug design and delivery.

Acknowledgements

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USA: +1 732 494 8660
UK: +44 (0)1604 542 500
China: +86 (0)21 6289 6060
Taiwan: +886 3 5600606
France: +33 (0)1 69 74 72 00
Italy: +39 06 51 59 22 1
India: +91 (80) 4127 3637
Brazil: +55 (0)11 2923 5400
Germany: +49 (0) 6251 8475 0
Japan: +81(75)313-8121
Singapore: +65 (6) 745-8300
Other: +33 (0)1 69 74 72 00