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Application News

OLS5100 3D Measuring Laser Microscope
AIRsight™ Infrared and Raman Microscope

Measurement of Microplastics in Mouse Lung Tissue by 3D Measuring Laser Microscope and Infrared and Raman Microscope

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User Benefits

- Searches and qualitative analysis of microplastics in biological tissue can be conducted easily by using a combination of a 3D measuring laser microscope (OLS5100) and Raman measurement with an infrared and Raman microscope (AIRsight).
- Screening for microplastics using the OLS5100 can be expected to shorten the time required for Raman measurements.
- Since the measurement region in Raman measurements using the AIRsight is on the order of a few µm, measurement of extremely small microplastics is possible.

■ Introduction

The environmental impact of microplastics (MPs), defined as plastic particles with sizes of no more than 5 mm, has become an issue. In recent years, there has also been concern that MPs in the air, soil or oceans may be ingested inadvertently by organisms and then be introduced into the human body through the food chain, and this may cause physical obstruction or damage of the feeding organ, gastrointestinal system, lungs, and other organs. Research is also underway to investigate the possibility that additives contained in MPs or harmful substances adsorbed on MPs in the natural environment may have adverse effects on the organs and trigger various diseases. Since the additives and harmful substances adsorbed on MPs differ depending on the type of plastic [1)], it is essential to identify not only the amount, but also the type of MPs in the body. However, due to the extremely small size of MPs in the body, it is difficult to investigate which MPs are present in the body, and in what amounts. Therefore, this article introduces an example in which MPs in the lung tissue of an experimental mouse after respiratory administration of simulated MPs were searched using Evident OLS5100 3D measuring laser microscope (Fig. 1), and a qualitative analysis to identify the type of plastic was carried out using an AIRsight infrared and Raman microscope (Fig. 2).

Fig. 1 Appearance of Evident OLS5100

Fig. 2 Appearance of IRXross™ (left) and AIRsight™ (right)

■ Analysis Samples

Polystyrene particles with a diameter of 3 µm were used as the simulated MPs in the measurement of the lung tissue of the experimental mouse after respiratory administration. Slices of the sampled lung tissue mounted on the slide glass were frozen and stored, and were used in the measurements after natural thawing and natural drying. Fig. 3 shows the condition of a sample observed with a stereoscopic microscope.

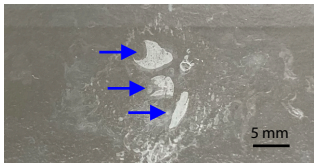

Fig. 3 Lung Tissue of Experimental Mouse on Slide Glass
(Parts Indicated by Arrows)

■ Screening of MPs by OLS5100

The OLS5100 is a high resolution laser scanning microscope (LSM) which uses laser light with a wavelength of 405 nm. LSM images and 3D images aligned to the same focal point over the entire field of view can be acquired quickly and simply by detecting the reflected light from the sample, and it is possible to measure the shape, dimensions, and other features of the sample. Particle analysis to obtain the characteristic quantities of particles, such as the particle diameter and height, from the high resolution LSM images and 3D images of powder samples is also possible.

Fig. 4 shows the LSM observation images of lung tissue samples A ((a) to (d)) and B ((e) to (h)) of the experimental mouse after respiratory administration of the simulated MPs.

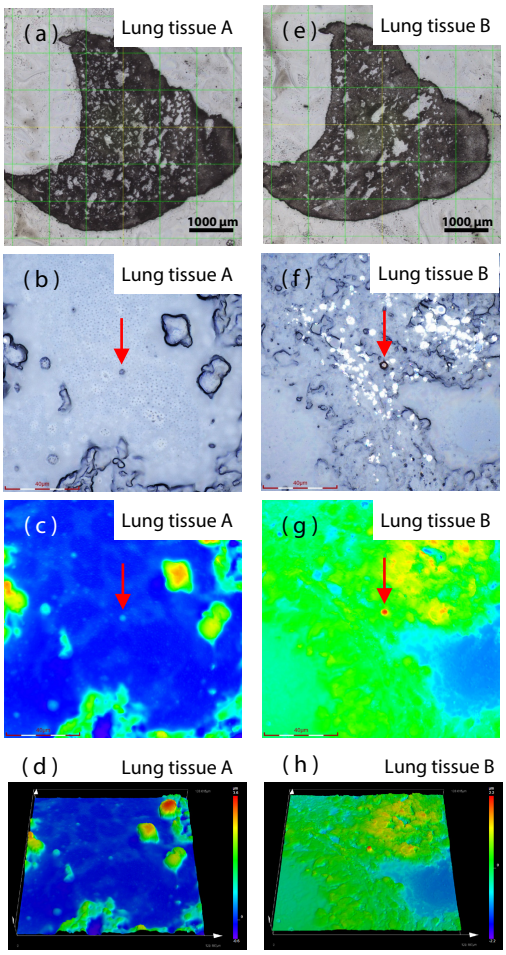

Fig. 4 LSM Images of Lung Tissue Samples A and B of Experimental Mouses After Respiratory Administration of Simulated MPs

(a) and (e) color images, (b) and (f) color images observed at high magnification, (c) and (g) 2-dimensional representations of height images observed at high magnification, and (d) and (h) 3-dimensional representations of height images observed at high magnification. The arrows in the figure indicate spherical substances.

Fig. 4 (a) shows a color image of lung tissue sample A observed at low magnification (5120 µm × 5120 µm). Fig. 4 (b) and (c) show 2-dimensional representations of the color image and height image of the central area in (a) observed at high magnification (129 µm × 128 µm). Fig. 4 (d) is a 3-dimensional representation of the height image observed at high magnification. Fig. 4 (e) to (h) are the results of the same observations of lung tissue sample B. In both lung tissue samples A and B, spherical substances with a diameter of approximately 3 µm were observed in the areas indicated by the arrows in the central part of the images observed at high magnification. Since it was possible that these spherical objects were the simulated MPs administered intratracheally to the mouse, a qualitative analysis was carried out by Raman measurement.

■ Raman Measurement by AIRsight Infrared and Raman Microscope

A qualitative analysis of two spherical substances, which were considered to be possible simulated MPs based on the results of observations with the OLS5100, was carried out by Raman measurements using the AIRsight. The proprietary wide-view camera installed in the AIRsight instrument as a standard feature makes it possible to observe a wide field of view up to a maximum of 10 mm × 13 mm, and also allows variable digital zooming of 5x. Because the reflecting objective mirror used for infrared measurements and the objective lens for Raman measurements share positional information with the wide-view camera, it is possible to switch smoothly between the reflecting objective mirror and the objective lens in order to set the same measurement position with no deviation of the field of view. This feature enables easy searching (screening) and measurement of the same field of view as that observed and photographed with the OLS5100.

Fig. 5 shows images acquired by AIRsight observation of lung tissue samples A and B of the experimental mouse with respiratory administration of the simulated MPs. (i) and (k) are wide-field camera images, and (j) and (l) are images acquired with the 100x objective lens. Table 1 shows the measurement conditions.

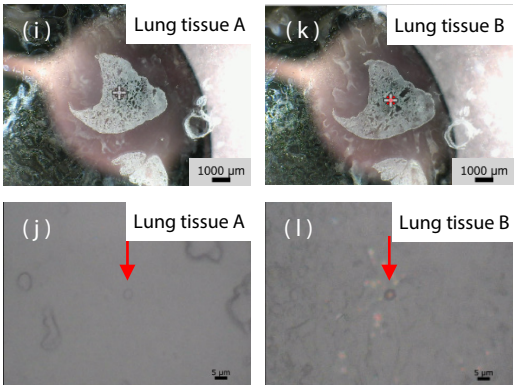

Fig. 5 AIRsight Images of Lung Tissue Samples A and B of Experimental Mouse After Respiratory Administration of Simulated MPs
(i) and (k) wide-field camera images and (j) and (l) images acquired with the 100x objective lens. The arrows in the figure indicate spherical substances.

Table 1 Measurement Conditions

Instruments	: IRXross, AIRsight
Accumulation	: 5 times
Exposure time	: 5.0 s
Objective lens	: 100x
Excitation wavelength	: 785 nm
Detector	: CCD

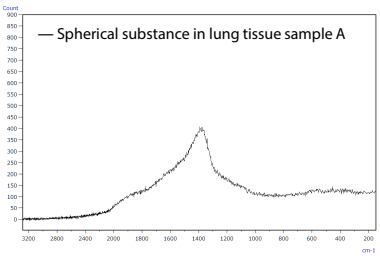

Fig. 6 Results of Raman Measurement of Lung Tissue Sample A of Experimental Mouse After Respiratory Administration of Simulated MPs

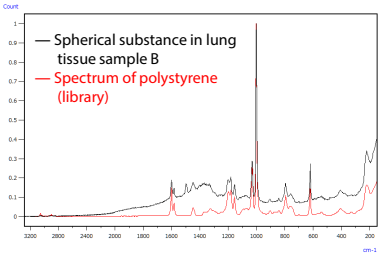

Fig. 7 Results of Raman Measurement of Lung Tissue Sample B of Experimental Mouse After Respiratory Administration of Simulated MPs

From the results in Figs. 6 and 7, the spherical substance in lung tissue B displayed good agreement with the library spectrum of polystyrene, indicating that the substance was the simulated MP administered to the mouse via the respiratory system. However, since no peaks were detected from the spherical substance in lung tissue A, it was clear that the substance was not the simulated MP. Looking at the results of LSM observation in Fig. 4, when compared with color image (b) of lung tissue A, the color tone of the spherical substance in color image (f) of lung tissue B is different from that of the surrounding lung tissue. Because the color tone in color images changes due to differences in the reflectivity of the observation target, it is thought that the reflectivity of the simulated MPs and the surrounding lung tissue were different, so their appearance was also different. Moreover, in comparison with the height images (c) and (d) of lung tissue A, in the height images (g) and (h) of lung tissue B, the spherical substance is shown in red, indicating that the spherical substance in lung tissue B is higher than the surrounding lung tissue. Considering these facts, it was found that, in LSM observation, it is possible to distinguish simulated MPs in lung tissue based on the information in color images and height images.

■ Conclusion

Screening of MPs using an OLS5100 and qualitative analysis by Raman measurement using an AIRsight were carried out for the lung tissue of an experimental mouse to which polystyrene (diameter: 3 µm) was administered via the respiratory tract as simulated MPs. In observation by the OLS5100, it was found that simulated MPs can be searched easily in a wide field of view by using the information in color images and height images. In Raman measurement by the AIRsight, the same field of view as in the OLS5100 observation could be searched easily, and it was possible to determine whether spherical substances were the simulated MPs or not. This technique is considered to be useful for searching and qualitative analysis of MPs in a wide range of biological tissues.

<Reference>

1) Lina Fu, Jing Li, Guoyu Wang, "Adsorption behavior of organic pollutants on microplastics," College of Forestry, Beijing Forestry University, Beijing, China, Ecotoxicology and Environmental Safety Volume 217, 112207, 1 July 2021.

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