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# Chemical Identification, Localization, and Counting of Ultrafine Particles in Complex Biological Matrices

There is an increasing public concern about the negative impact of ultrafine particles (UFPs) on human health. UFPs are aerosols with a diameter of 100 nm or less that are able to penetrate biological barriers, contribute the highest number of particles in the atmosphere, and have the largest surface area promoting the adsorption of additional hazardous substances [1]. Here, we demonstrate advanced analytical workflows based on correlative spectroscopy (spontaneous and stimulated Raman, X-ray fluorescence (XRF)) and microscopy (optical, electron, fluorescence) techniques using relocalization technologies from Horiba (nanoGPS and Particle Finder) [2]. These complementary methods were first applied to standard plastic particles, in this case polystyrene with the smallest diameter of 50 nm for the initial development of workflows and then to real, environmental particles (smog, break dust, and tyre wear), all being part of UFPs pollution. Investigations of chemical and elemental composition by Raman and XRF revealed a mixture of carbon- and metallic-based nanoparticles, while high-resolution microscopy showed both single and clustered UFPs. We found amorphous carbon in all environmental samples, mainly S, Na, Si, Ca, Fe (~84.6, 6.5, 3.4, 1.6, 1.1 wt%) on the smog filters, and mostly Fe, Si, Al, Ti, S, Zn, Ca, Sn (~85.3, 1.9, 1.8, 1.5, 1.4, 1.3, 1.3, 1.1 wt%) in the break dust, largely as oxide compounds. This detailed precharacterization of the UFPs allowed us to efficiently detect them inside intricate biological matrices at cell, organ tissue, and animal levels. We performed extensive exposure experiments on in-vivo human podocytes and Calu-3 cells as representative kidney and lung cells prone to accumulation of particles during lifetime, model mice similar to human ulcerative colitis, and model zebrafishes resembling human membranous glomerulonephritis affecting kidneys. Distinct functional and gene expression biomarkers were observed for UFP-treated cells compared to non-treated controls. Mice with an inflamed gastrointestinal tract showed an impaired mucosal barrier function facilitating particles to enter in the bloodstream, thereby spreading and accumulating in all organs. Zebrafishes also showed the presence of UFPs in all organs implying uptake through the digestive system rather via skin. Thus, Raman integrated in correlative workflows supported by machine learning algorithms can be used to correctly estimate the exposure level and health effects of UFPs.

## References

[1] Kwon et al., Experimental & Molecular Medicine, 52 (2020) 318–328
[2] G. Sarau et al., Horiba Readout, 54 (2020) 23-32

## Figures

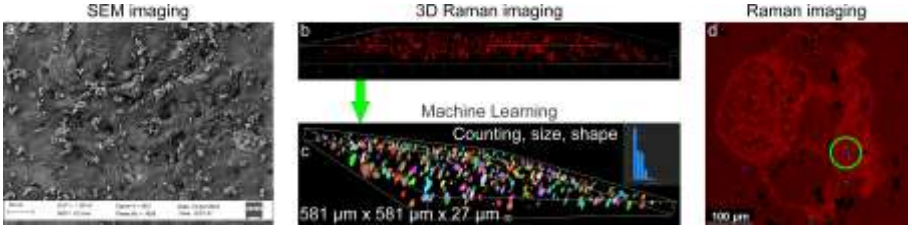


**Figure 1:** Example of spectroscopy and microscopy workflow applied to lung cells exposed to UFPs (a, b) followed by machine learning particle analysis (c). Accumulation of UFPs in zebrafish organ tissue (d).