Why Nanoplastics Researchers Use Enhanced Darkfield Hyperspectral Microscopy

Microplastics and nanoplastics currently receive more attention than almost any other area of environmental toxicology research. This attention is well founded when considering in 2018 alone, an estimated 389 million tons of plastic waste was generated.[1] Given the scale of this problem, and the lack of insight regarding how micro and nanoscale plastics affect the food chain and environment, quality research and highly effective research tools are critical components in the efforts to address this important issue.

From a research perspective, there are two primary areas of focus related to nanoplastics. The first is an effort to gain an accurate understanding of the scale, scope and types polymers in the environment and the food chain at both the micro and nano scale. The second area is focused on understanding how the exposure to different types, shapes and sizes of polymers can affect aquatic organisms, plants and humans.

While this app note is primarily focused on the second area of understanding the effect of nanoplastics and environmental exposure, it is important to first acknowledge the need to determine where nanoplastics are present in the environment. This effort requires effective collection and quantitation techniques to learn not only where plastics are found, but to accurately identify their size, shape and type, and which are most pervasive. For this area of research, highly quantitative analysis techniques are required using instruments such as ICP-MS, SEM-EDS, FTIR and Raman. Each of these techniques can provide accurate quantitative data regarding the speciation of plastics in a wide range of environments. However, these techniques can also be sample destructive, limited in their spatial imaging capabilities, and often require specialized sample preparation.

Once these quantitative systems have conclusively confirmed the presence of nanoplastics in specific areas of the environment, the focus then shifts to understanding how the exposure to different types, shapes and sizes of polymers can affect aquatic organisms, plants and humans. This research is typically conducted in highly structured experiments where all variables except the plastics exposure are kept consistent versus a negative control. In these controlled experiments, plastics of known sizes, shapes and chemistries are exposed to these specific environmental targets to understand how the plastics interact with the target environment and how said environment is affected by the exposure. For these controlled exposure experiments, the ability to conduct rapid, high contrast imaging and spectral analysis can be critical to understand where the plastics localize in the different living organisms, how long plastics remain and the overall impact on the environment.

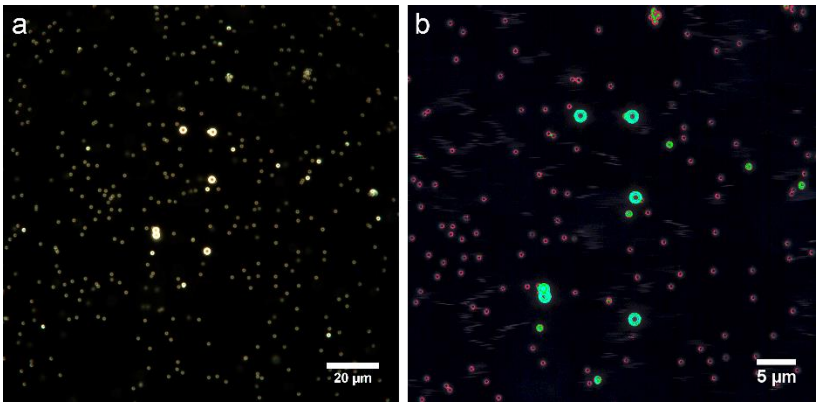

Figure 1 – Enhanced dark-field image (a) and hyperspectral mapping delineation (b) of 1 um polystyrene (PS), 1 um polymethyl methacrylate (PMMA) and 2 um melamine formaldehyde (MF) microplastics mixture in aqueous solution.

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CytoViva's Enhanced Darkfield Hyperspectral (EDF-HSI) microscopy is a critically important tool in controlled experiments involving nanoplastics exposure in the environment. CytoViva's patented enhanced darkfield microscope optics create high signal-to-noise images based on light scattered by these nano-plastics and their environment. The optical imaging of nanoplastics can be conducted without any special staining, immunofluorescence or other manipulation of the sample. When the CytoViva microscope is further equipped with CytoViva's hyperspectral imaging system, the optical spectrum from 400 nm-1,000 nm or 900 nm-1,700 nm can be recorded in every single pixel of the image. The pixel resolution of a hyperspectral image pixel can be as much 1,400 x 1,400 with pixel sizes below 100 nm. This enables the recording and spectral mapping of these nanoplastics down to the single nanoparticle level.

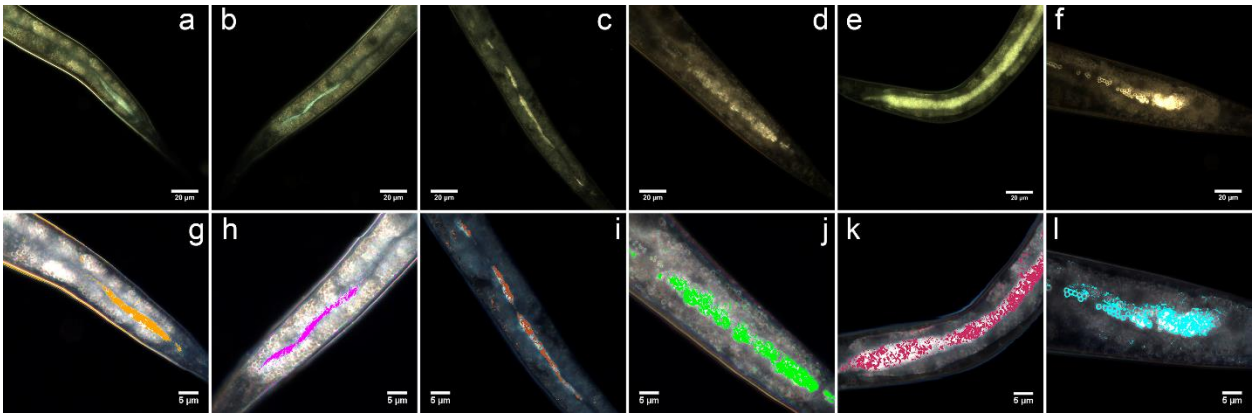

Figure 2 – Enhanced dark-field microscopy images (top row) and corresponding hyperspectral images merged with spectral angle mapper (SAM) algorithm-based maps (bottom row, simulated color) demonstrating the distribution of nano- and microplastics (pure) in *C. elegans* young adult nematodes: (a and g) – PS-100 nm; (b and h) – PS-200 nm; (c and i) – PS-500 nm; (d and j) – PS-1 µm; (e and k) – PMMA-1 µm; (f and l) – MF-2 µm.

A methods papers demonstrating the efficacy of CytoViva's EDF-HSI microscopy for nanoplastics research was recently published in the Environmental Pollution journal entitled Dark-field Hyperspectral Microscopy for Label-free Microplastics and Nanoplastics Detection and Identification in vivo: A Caenorhabditis Elegans Study.[2] This paper was written by researchers from the Institute of Fundamental Medicine and Biology at Kazan Federal University. This laboratory, led by Dr. Rawil Fakhrullin, has been a prolific publisher in the area of nanotoxicology and has written multiple methods papers on the use of the CytoViva EDF-HSI microscopy technology.

In this paper, the authors demonstrate the ability to optically observe, spectrally characterize and spectrally map a number of chemically different micro and nano scale plastics confined within the intestines of optically transparent live invertebrates Caenorhabditis elegans. The figures above and below were contributed by Dr. Fakhrullin et al., and are from similar work to the referenced publication. These images illustrate the ability to identify and differentiate chemically different micro and nano scale sized plastics particles in aqueous solution and in C. *elegans*.

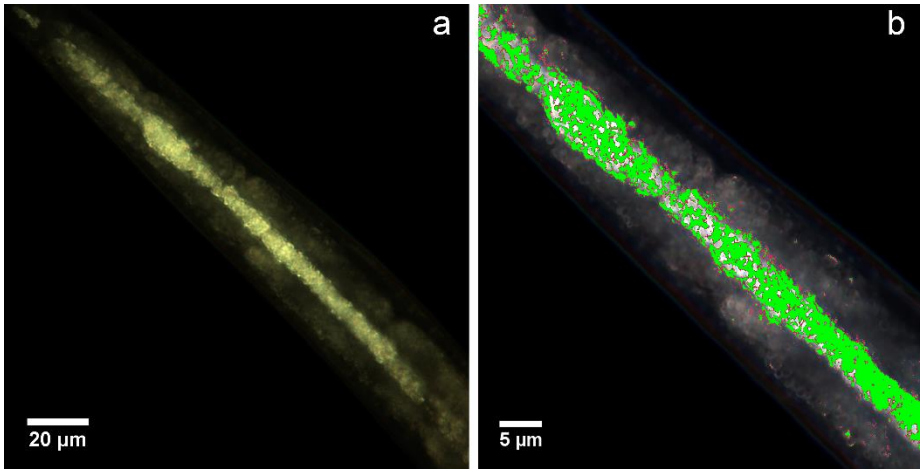

Figure 3 – (a) Enhanced darkfield image and (b) corresponding hyperspectral images merged with SAM algorithm-based maps demonstrating the distribution of microplastics mixed in *C. elegans* young adult nematodes: 80% PS (mapped green) and 20% PMMA (mapped red).

Figure 1 above illustrates 1 um sized polystyrene, 1 um sized polymethyl methacrylate and 2 um sized melamine formaldehyde microplastics that were mixed together in an aqueous solution. A spectral library of each plastic, when isolated, was created and then applied to the mixed sample. This enables the different species of plastics to each be identified in the mixed format by spectrally mapping each a different color.

Figure 2 above illustrates optical enhanced darkfield and hyperspectral microscopy images of *C. elegans* exposed to a range of different sizes and different types of micro and nano scale plastics. Using the spectral angle mapping algorithm, the nanoplastics ingested by the *C. elegans* are mapped throughout the organism, with each different plastic exhibiting a different simulated color.

In Figure 3, the research team illustrates the ability to spectrally map different types of plastics exposed to the *C. elegans* in different concentrations (80% PS and 20% PMMA). The corresponding spectral mapping of the plastics is consistent with the concentration amounts, with the PS particles predominately mapping in the sample image.

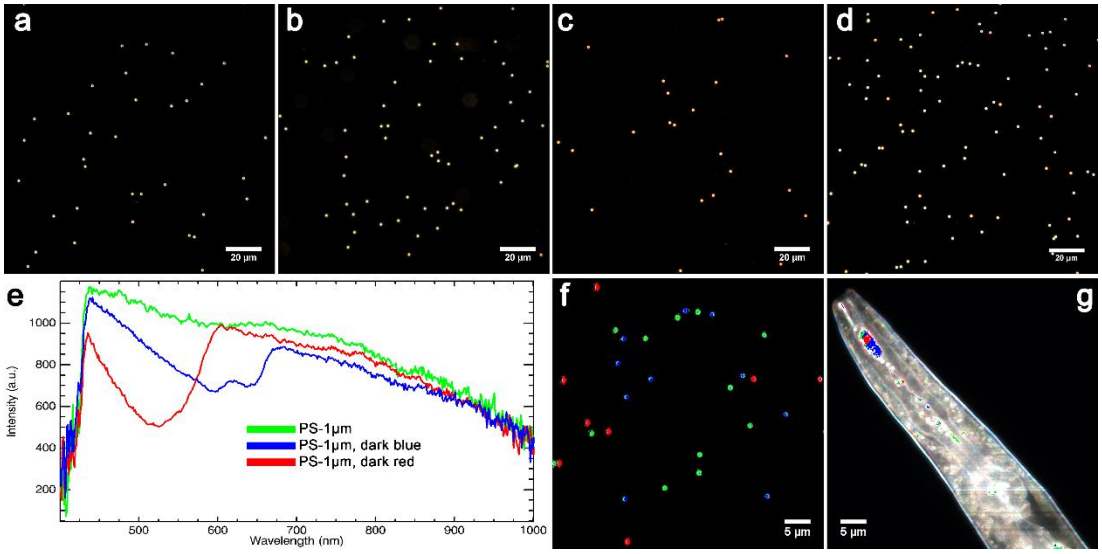

Figure 4 – Dark-field images of 1 µm polystyrene particles (a), and pigmented 1 µm polystyrene particles: dark blue (b) and dark red (c). Spectral signatures of polystyrene microspheres of different colors (e). Dark-field imaging (d) and hyperspectral mapping (f) of a mixture of PS-1µm, PS-1µmR and PS-1µmB in water. A hyperspectral image merged with the map showing the distribution of PS-1 µm (green), PS-1 µm red dark (red) and PS-1 µm blue dark (blue) in a young adult nematode C. *elegans* (g).

Additives like colorants can affect the potential toxicity of plastics as they can contain heavy metals and contaminants.[3] As such, it can be helpful to study these materials based on color or other non-polymer speciation processes. Optical hyperspectral microscopy can differentiate plastics based on differences in their color. The example images above illustrate three differently colored polystyrene particles mixed together in aqueous solution and ingested by a *C. elegans*. The spectral differences based on the color are shown along with spectral mapping for each of these color differences in water and internalized by the *C. elegans*.

While no single instrument can provide the complete answer for required research in areas such as nanoplastics, the examples shown above illustrate how CytoViva's Enhanced Darkfield Hyperspectral microcopy can be an effective tool for studying these materials in controlled experiments where significant insight can be elucidated from high contrast imagery without any specialized sample preparation. To understand how CytoViva technology can advance your research in nanoplastics or related experiments, please contact us at info@cytoviva.com or visit us at www.cytoviva.com to learn more.

References

[1] PlasticsEurope. Plastics the Facts—2019; PlasticsEurope: Brussels, Belgium, 2020.

[2] Läysän Nigamatzyanova, Rawil Fakhrullin, Dark-field hyperspectral microscopy for label-free microplastics and nanoplastics detection and identification in vivo: A Caenorhabditis elegans study, Environmental Pollution, Volume 271, 2021, 116337, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2020.116337

[3] Maxi B. Paula, Valerie Stock, Julia Cara-Carmona, Elisa Lisicki, Sofiya Shopova, Valérie Fessard, Albert Braeuning, Holger Sieg and Linda Böhmerta: (2020) Micro and nanoplastics – current state of knowledge with the focus on oral uptake and toxicity Nanoscale Adv. 2 10 4350-4367 http://dx.doi.org/10.1039/D0NA00539H